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Endocrine Mediation in Termination of Pupal Diapause in *Antheraea mylitta* Drury (Lepidoptera: Saturniidae)

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Abstract: Removal of brain from variously aged diapausing pupa induced developmental arrest. Implantation of day 1 brain of non–diapausing pupa into 195 days old dauer pupa evoked adult development in 48% insects indicated the dependency on brain for diapause termination in *A. mylitta* pupa. Injection of ecdysterone into diapausing pupae resulted in age–dependent responses. Low doses of 0.5μg or 1μg per gram body weight did not induce adult development in young pupae but induced pupal–adult transformation when treated on or after 90 days. All the 90 days old diapausing pupae treated with 2μg or 3μg ecdysterone transformed into adults within 27 to 28 days. Ecdysterone treatments to brainless diapausing pupae also initiated adult development. These observations suggest that pupal diapause in *A. mylitta* is a result of the deficiency of ecdysterone due to the lack of brain activation of prothoracic glands.

Keywords: *Antheraea mylitta*, Pupal diapause, Brain–prothoracic gland system

INTRODUCTION

Diapause is a state in which development is suppressed and metabolism decreased in order to survive during unfavourable environmental conditions. Environmental factors involved in induction and termination of diapause include temperature, photoperiod, moisture and diet of which the photoperiod is considered as the major factor (Beck, 1968; Saunders, 1981). Pupal diapause occurs as a result of cessation of brain prothoracicotropic hormone (PTTH) secretion leading to an ecdysone deficiency (Gilbert and King, 1973; Denlinger 1985).

Diapausing pupae are refractory to diapause termination until the brain regains its potency to translocate PTTH. The environmental cues act on the brain to modulate PTTH secretion and thereby to control the termination of pupal diapause (Williams, 1956; Williams and Adkisson, 1964). The PTTH regulates prothoracic gland activity using cyclic AMP as second messenger (Smith *et al.* 1986; Smith 1993). Post–diapause period is characterised by the appearance of large peaks of ecdysteroid titre in haemolymph which causes adult development (Bodnaryk, 1985; Bowen, *et al.* 1985).

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An induced increase of ecdysteroid titre in the haemolymph by injection into diapausing pupa seems to evoke imaginal differentiation in some insect species (Gibbs, 1976; Browning, 1981; Denlinger, 1985).

The tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) exhibits bivoltinism or trivoltinism depending on the environmental conditions. Growth rate affects timing of release of PTTH in larval instars which in turn influence voltinism (Pradeep, *et al.* 1995). After 1 or 2 non–diapausing generations, larvae enter diapause at pupation which extends for 6–7 months. Termination of pupal diapause in *A. mylitta* is influenced by temperature and photoperiod (Jolly, *et al.* 1971), but how the neuroendocrine system respond to environmental factors to induce diapause termination and adult differentiation is not known. Present investigation is to explore the possible role of brain–prothoracic gland system in maintenance and termination of diapause in the *A. mylitta* pupa using brain extirpation and implantation techniques and by 20–hydroxyecdysone treatments.

MATERIALS AND METHODS

A. mylitta larvae of bivoltine generation were reared on fresh leaves of the host plant, *Terminalia tomentosa* under short photoperiod (11L : 13D) and low temperature (21 ± 4 °C) which induce pupal diapause (Jolly *et al.* 1974). Newly ecdysed (day 0) pupae were obtained from 15–20 days old cocoons of the diapause–destined larvae and were used for experiments at various ages.

Brain was extirpated from ether–anaesthetised pupae through a small incision made at the brain window present on the dorsum of the head. The day of brain extirpation was considered as day 0 of dauer pupae. Brain of day 1 non–diapausing pupa was dissected out in ice–cold Ringer solution and implanted immediately into 195 days old dauer pupa through a small slit made in between 5th and 6th abdominal tergum. In control insects, only wound was made. The wounds were sealed with melted paraffin wax in both control and experimental insects.

Ecdysterone (20–hydroxyecdysone, Sigma Chem. Co., U. S. A) was dissolved in 10% ethanol and diluted with water. Appropriate dose per gram body weight of pupa was given as single injection in between 4th and 5th abdominal tergal plates, using a Hamilton microlitre syringe. Control insects were injected with an equivalent volume of 10% ethanol only.

RESULTS AND DISCUSSION

In normal insects kept without any experimental manipulations, the pupal diapause was prolonged for 6–7 months and adult emergence occurred after 195.98 ± 8.6 days of pupation whereas non–diapausing pupae completed pupal–adult transformation within 22.33 ± 3.18 days. Brain was extirpated from variously aged diapausing pupae. Large proportion of the debrained pupae failed to undergo adult development and transformed to dauer pupae and died after surviving for 9–10 months. Control insects underwent pupal–adult transformation along with normal insects (Fig. 1). Brain removal blocked the ability for diapause termination. This revealed that presence of brain was required during entire period of diapause for termination. Such long dependency on the brain until the very end of diapause was recorded in *Hyalophora*

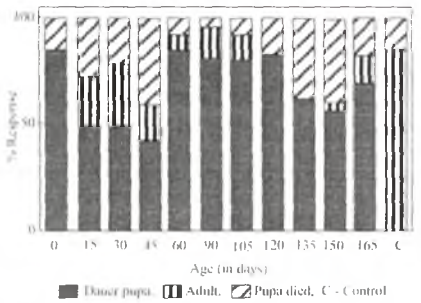

Fig. 1.

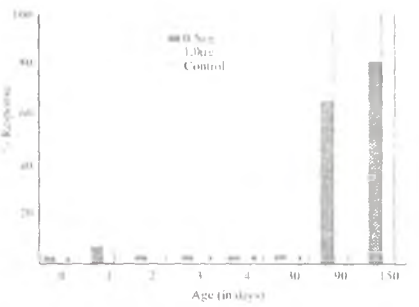

Fig. 2

Fig. 1. Response of diapausing pupa of *A. mylitta* to debraining at different ages. $n = 30$ each.
Fig. 2. Per cent of diapausing pupae of *A. mylitta* transformed to adults after injection with ecdysterone and control. $n = 30$ each.

cecropia (Williams, 1956). In a related species *Antheraea polyphemus* and several other insects, development was independent of brain after specific periods of diapause (Mc Daniel and Berry, 1967; Denlinger 1985). Debraining of non–diapausing pupae inhibited adult development in 94% insects when extirpated on or before day 2 (Table 1) showed that presence of brain was required during this period to initiate adult development. Implantation of single brain of non–diapausing day 1 pupa into 195 days old dauer pupa initiated adult development in 47.5% insects (Table 2) indicated that prothoracic gland remained competent long enough for the brain hormone to activate it (Browning, 1981). Since the brain of non–diapausing pupa was already programmed for development, it might have override the diapause and initiated adult development (Yagi and Honda, 1977). It is well documented that brain neurosecretory cell secretes PTTH which stimulates prothoracic gland causes an elevated ecdysone titre in haemolymph during critical periods of pupal development in order to promote adult differentiation (Denlinger, 1985).

Table: 1 Effect of debraining of non–diapausing pupa of *A. mylitta*

Age (in days)	*n*	% Morality	% insects		Time took for completion of adult development [in days (Mean ± S. E.)]
			initiated development	transformed to dauer pupa	
0	20	15	–	85.0	–
1	30	–	3.30	96.70	54*
2	16	–	6.25	93.75	61*

*Only one insect each

Treatments of ecdysterone at a concentration of 0.5μg and 1.0μg per g body weight to diapausing pupae resulted in age–dependent responses in evoking developmental events (Fig. 2). Absence of adult differentiation in early aged treated pupae might be due to non–persistence of low doses of ecdysterone for a sufficiently long period to permit adult morphogenesis, possibly due to ecdysone degrading mechanisms operative during development (Ohtaki, *et al.* 1968; Karlson and Bode, 1969). When

90 days old diapausing pupae were treated with various doses of ecdysterone, high doses evoked adult development in all the test insects within 28 ± 0.5 days (Table 3). Control insects continued in diapause and adult emergence occurred after 114 ± 6 days. Ecdysterone induced termination of diapause and initiation of adult development in *A. mylitta* confirms with earlier reports (Bodnaryk, 1977; Denlinger, 1985). The exogenous hormone supposed to have exerted a positive feed back effect on the prothoracic glands to secrete a threshold level of the hormone in order to facilitate successful pupal-adult transformation. (Kirmura and Kobayashi 1975; Demlinger, 1985). Thus diapausing pupa of *A. mylitta* is competent to initiate adult development in the presence of sufficient circulating ecdysteroids. Ecdysteroid titre is found to have increased significantly within a few days after pupation in developing insects but stayed at basal level in diapausing insects (Bowen, *et al.* 1985; Bodnaryk, 1986; Richards *et al.* 1987).

Table: 2 Effects of implantation of brain into debrained pupa of *A. mylitta*

Experiment	Age of pupa (in days)	*n*	% Mortality till control emergence	% Insects initiated adult development	% Insects transformed to dauer pupa	Time took for completion of adult development after experiment [in days (Mean ± S.E)]
Brain extirpation	90	30	04.00	14.0	82	130.00 ± 2.31
Brain extirpation + Brain implantation	90 / 285	40	32.50	47.5	20	56.71 ± 2.229

Table: 3 Effects of treatments of 20–hydroxyecdysone to normal and debrained pupae of *A. mylitta*

Dose (μg/g. body wt.)	*n*	% Mortality	% Surviving insects initiated adult development	% Surviving insects transformed to dauer pupa	Time took for completion of adult development [in days (Mean ± S.E)]
Normal 2	30	–	100.00	00.00	28.27 ± 0.267
3	30	–	100.00	00.00	28.40 ± 0.399
Debrained 2	30	–	90.00	10.00	26.52 ± 0.328
3	30	26.67	96.67	03.33	28.30 ± 0.562
10 μl 10% ethanol	36	03.70	00.00	96.30	–

Injections of 2μg or 3μg ecdysterone to 40 days old dauer pupae induced adult development in 90–97% insects. All the control brainless insects continued as dauer pupae (Table 3). Obviously, ecdysone deficiency due to absence of brain activation of prothoracic glands is the reason for continuation of diapause and formation of dauer pupa in *A. mylitta*. Presence of PTTH from brain was necessary for the prothoracic

gland to become competent to respond to the stimulus for development (Bowen, *et al.* 1984; Smith, *et al.* 1986).

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REFERENCES

Beck S. D. (1968) *Insect photoperiodism*. Academic Press, New York.

Bodnaryk R. P. (1977) Stages of diapause development in the pupa of *Mamestra configurata* based on the β–ecdysone sensitivity index. *J. Insect Physiol.* **23**: 537–542.

Bodnaryk, R. P. (1985) Ecdysteroid levels during post-diapause development and 20-hydroxyecdysone-induced development in male pupae of *Mamestra configurata* Wlk. *J. Insect Physiol.* **31**: 53–58.

Bodnaryk, R. P. (1986) Ecdysteroid hormone levels in the pupa of the bertha armyworm, *Mamestra configurata* Wlk., during spermatogenesis. *J. Insect Physiol.* **32**: 931–935.

Bowen M. F., Bollenbacher, W. E. and Gilbert, L. I. (1984) *In Vitro* studies on the role of the brain and prothoracic glands in the pupal diapause of *Manduca sexta*. *J. exp. Biol.* **108**: 9–24.

Bowen M. F., Irish, R., Whisenton, L. R., Granger, N. A., Gilbert, L. I. and Bollenbacher, W. E. (1985) Endocrine events during prediapause and non–diapause larval–pupal development of the tobacco hornworm, *Manduca sexta*. *J. Insect Physiol.* **31**: 83–90.

Browning T. O. (1981) Ecdysteroids and diapause in pupae of *Heliothis punctiger*. *J. Insect Physiol.* **27**: 715–719.

Denlinger D. L. (1985) Hormonal control of diapause. In: *Comprehensive Insect Physiology Biochemistry and Pharmacology*. G. A. Kerkut and L. I. Gilbert Eds., Pergamon Press, Oxford, **8**: 353–412.

Gibbs D. (1976) The initiation of adult development in *Sarcophaga argyrostoma* by β–ecdysone. *J. Insect Physiol.* **22**: 1195–1200.

Gilbert L. I. and King, D. S. (1973) Physiology of growth and development: endocrine aspects. In: *The Physiology of Insecta* 2 edn. M. Rockstein Ed., Academic Press, New York. 1: 249–370.

Jolly M. S., Sinha, S. S. and Razdan, J. L. (1971) Influence of temperature and photoperiod on termination of pupal diapause in the tasar silkworm, *Antheraea mylitta*. *J. Insect Physiol.* **17**: 753–760.

Jolly M. S., Sen, S. K. and Ahsan, M. M. (1974) *Tasar Culture*, Ambika Publishers, Bombay.

Karlson P. and Bode, C. (1969) Die inaktivierung des Ecdysons bei der Schmeiss fliege. *Calliphora erythrocephala* Meigen. *J. Insect Physiol.* **15**: 111–118.

Kimura S. and Kobayashi, M. (1975) Prothoracicotropic action of ecdysone analogues in *Bombyx mori*. *J. Insect Physiol.* **21**: 417–421.

Mc Daniel C. N. and Berry, S. J. (1967) Activation of the prothoracic glands of *Antheraea polyphemus*. *Nature*. **214**: 1032–1034.

Ohtaki T., Milkman, R. D. and Williams, C. M. (1968) Dynamics of ecdysone secretion and action in the fleshfly *Sarcophaga peregrina*. *Biol. Bull. Woods Hole* **135**: 322–334.

Pradeep A. R., Singh, M. K. and Sinha, S. S. (1995) Growth and its relation with prothoracicotropic hormone release during larval instars of *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *Phytophaga* **7**: 65–72.

Richards D. S., Warren, J. T., Saunders, D. S. and Gilbert, L. I. (1987) Haemolymph ecdysteroid titres in diapause and non–diapause–destined larvae and pupae of *Sarcophaga argyrostoma*. *J. Insect Physiol.* **33**: 115–122.

Saunders D. S. (1981) Insect Photoperiodism: The clock and the counter. *Physiol. Entomol.* **6**: 99–116.

Smith W. A. (1993) Second messengers and the action of prothoracicotropic hormone in *Manduca sexta*. *Am. Zool.* **33**: 330–339.

Smith W. A., Bowen, M. F., Bollenbacher, W. E. and Gilbert, L. I. (1986) Cellular changes in the prothoracic glands of diapausing pupa of *Manduca sexta*. *J. Exp. Biol.* **120**: 131–142.

Williams, C. M. (1956) Physiology of insect diapause. X. An endocrine mechanism for the influence of temperature on the diapausing pupa of the cecropia silkworm. *Biol. Bull.* **110**: 201–218.

Williams C. M. and Adkisson, P. L. (1964) Physiology of insect diapause. XIV. An endocrine mechanism for the photoperiodic control of pupal diapause in the oak silkworm *Antheraea pernyi*. *Biol. Bull.* **127**: 511–525.

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Effectiveness of Seven Vegetable Oils Against *Callosobruchus chinensis* L. in Pigeon Pea *Cajanus cajan* L.

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Abstract: The effects of seven available vegetable oils at 0.4, 0.6 and 0.8% w/w concentrations/100 grams of pigeon pea seed on oviposition of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) were studied at 30±3° and 75±6% RH. Observations were taken on 1, 15, 30 and 45 days after treatment. The seven vegetable oils tested viz *Cymbopogan citratus, Bassia longifolia, Ricinus communis, Cocos nucifera, Arachis hypogea, Glycine max* and *Azadirachta indica* significantly effected on oviposition at 3 concentrations tested at one day after treatment. *C. citratus, R. communis* and *B. longifolia* at 0.8% concentration were found to reduce oviposition significantly compared to the control. All seven vegetable oils tested did not effect the germination of seeds significantly.

Keywords: Vegetable oils; *Callosobruchus chinensis*, Pigeon pea effectiveness.

INTRODUCTION

Pigeon pea (*Cajanus cajan* L.) is a pulse crop now rapidly gaining momentum in Sri Lanka as a suitable crop for cultivation in the dry zone both as a vegetable and as a source of vegetable proteins. This crop was subjected to significant losses from a pod borer complex in early seventies in the field (Subasinghe and Fellows, 1978) and the farmers were forced to abandon the pigeon pea cultivation. There is renewed interest in pigeon pea cultivation in the dry zone again in Sri Lanka with active support from International Crops Research Institute for Semi Arid Tropics (ICRISAT) in Hyderabad, India. However the seeds of pigeon pea could also suffer qualitative and quantitative losses from the attack from the pulse beetle *Callosobruchus* spp. during storage. There are no reports to our knowledge on the use of vegetable oils in pigeon pea in Sri Lanka to protect it from *Callosobruchus* attack.

Mixing with plant oils is an ancient Indian and African method of protecting grains against insect attack (Pereira, 1983). In Sri Lanka too this is a traditional method used by small scale farmer and in households. Varma and Pandey (1978) showed that groundnut and other oils applied at 0.3% w/w (3 g oil/100 g of seed) gave complete

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protection to greeengram *Vigna radiata* (L. Wilczek) against *C. maculatus* in the laboratory. Singh *et al* (1978) reported that groundnut oil applied to cowpea had no effect on mortality or longevity of the adult *C. maculatus*. Hill and Schoonhoven (1981) found that palm oil killed adult *C. maculatus*. Therefore it is clear that there have been great discrepancies about the extent of protection offered by these oils against stored product insects (Pereira, 1983, and Schoonhoven, 1978). Van Huis (1991) too supported this view by reporting that there have been various reports of oils causing reduced oviposition and higher egg and adult mortality, but all do not agree. Therefore the objective of the present study was to evaluate the effect of seven available vegetable oils used by the majority of Sri Lankans against *C. chinensis* on pigeon pea.

MATERIALS AND METHODS

A culture of *Callosobruchus chinensis* L. was maintained using the procedure described by Strong *et al* (1968). The pulse beetle *C. chinensis* was identified by the key given by Raina (1970). The experiment was conducted at the laboratories of the Department of Agricultural Biology, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka during 1993-94 period.

One kg of Pigeon pea was mixed with seven vegetable oils viz: *Cymbopogan citratus* Stapt. (lemon grass), *Bassia longifolia* L. (mee), *Ricinus communis* L. (castor), *Cocos nucifera* L. (coconut), *Arachis hypogea* L. (groundnut), *Glycine max* L. Merr. (soybean), *Azadirachta indica* A. Juss (neem) in glass jars at concentrations 0.4, 0.6, 0.8 parts/100 parts of pigeon pea seed (w/w). Fifteen grams of treated seed at 3 different concentrations were placed in a glass tube 4" × 1") and a mechanical shaker was used for mixing the seed thoroughly. In each tube, a freshly emerged (0-24 hr) pair of *C. chinensis* was released at 0, 14, 29 and 44 days of treatment at 3 concentrations tested. These treatments were replicated six times. The oviposition of the female beetle was observed at 24 hours after releasing the pair of adults at 1, 15, 30 and 45 days after treatment and the mean number of eggs laid were counted.

Seed germination was also tested 45 days after treatment. These studies were conducted at room temperatures from 27-33°C with 60-80% RH. The data were analyzed using the General Linear Models Procedure of the SAS software package.

RESULTS

All the seven oils tested in this study significantly inhibited oviposition at 3 concentrations tested when compared with control. The *C. citratus* (Lemon grass) oil was the most effective at 0.8 concentrations in comparison to other oils tested (Table 1). The oils *C. citratus*, *Ricinus communis* (Castor), *B. longifolia* (Mee) and *C. nucifera* (Coconut) at 0.8 concentrations were found to be more effective than other oil concentrations tested (Table 1). Among the oils tested except for *G. max* and *A. indica*, 0.8 concentration was the most effective. For *G. max* and *A. indica* the most effective concentration was 0.6 w/w and for *G. max* 0.8 concentration recorded the highest mean oviposition compared to other two concentrations tested.

Table 1. Effect of vegetable oils on oviposition of one pair of *C. chinensis*

Vegetable oil	Dosage (w/w)	Mean oviposition
Cymbopogan citratus	0.4	6.08 H
	0.6	5.12 M
	0.8	4.20 Q
Bassia longifolia	0.4	5.51 L
	0.6	5.74 I
	0.8	4.40 O
Ricinus communis	0.4	6.39 E
	0.6	5.11 M
	0.8	4.27 P
Cocos nucifera	0.4	6.74 D
	0.6	5.66 J
	0.8	4.96 N
Arachis hypogea	0.4	7.14 C
	0.6	6.44 E
	0.8	6.25 F
Glycine max	0.4	6.25 F
	0.6	6.08 H
	0.8	7.37 B
Azadirachta indica	0.4	6.73 D
	0.6	5.57 K
	0.8	6.18 G
Control		10.48 A

Means with same letter are not significantly different at 5% level.

The effectiveness for the seven vegetable oils tested decreased with time period of storage (Table 2).

Table 2. Effect of days after treatment (DAT) on oviposition of one pair of *C. chinensis*

DAT	Mean oviposition
1	3.69 D
15	6.25 C
30	7.03 B
45	7.15 A

Means with same letter are not significantly different at 5% level.

All the vegetable oils tested did not affect the seed germination significantly (Table 3).

Table 3. The effect of vegetable oils on germination of seeds of pigeon pea

Vegetable oil	Dosage (w/w)	Mean germination
Cymbopogan citratus	0.4	84.0
	0.6	82.0
	0.8	85.0
Bassia longifolia	0.4	80.0
	0.6	83.7
	0.8	81.0
Ricinus communis	0.4	85.0
	0.6	83.7
	0.8	80.0
Cocos nucifera	0.4	81.3
	0.6	80.0
	0.8	82.0
Arachis hypogea	0.4	86.0
	0.6	81.0
	0.8	80.0
Glycine max	0.4	88.0
	0.6	81.0
	0.8	80.0
Azadirachta indica	0.4	85.0
	0.6	84.5
	0.8	81.3
Control		89.0

DISCUSSION

All the vegetable oils tested significantly inhibited oviposition at 1 day after treatment (DAT) and 4 oils tested in this study, *C. citratus*, *R. communis*, *B. longifolia* and *C. nucifera* were the most effective at 0.8% concentration. Ovicidal properties of vegetable oils like groundnut, coconut has already been reported (Mummigatti and Ragunathan, 1977, Singh *et al.* 1978). The mechanism involved in the protection of oil treated seeds in unclear. Majority of authors have reported that ovicidal nature of the oils were due to physical nature of oils to block the oxygen supply for the developing embryo or due to the toxicity of some constituents of the oils tested. Chander and Ahmed (1986) reported that *B. longifolia* oil was ovicidal at lower concentrations but a higher concentrations of 5 ml/kg seed, it might have exhibited insecticidal activity. However factors other than oxygen starvation probably also play a role in their mode of action (Schonhoven, 1978). Hence findings in Sri Lanka on neem, castor, groundnut oils

were in conformity with those of Sangappa (1977), Khaire *et al.* (1992) and Chander and Ahmed (1986). Singh *et al.* (1978) reported that castor oil at 8 mg/kg provided complete protection against *C. maculatus*. Castor oil on *Vigna radiata* L. seeds at 10 ml/kg provided complete control of *C. chinensis* for 18 months without affecting seed viability (Babu *et al.*, 1989). In addition to reduced oviposition, higher adult and larval mortality due to vegetable oils have also been reported (Van Huis, 1991). He also reported that vegetable oils did not affect the viability, palatability, cooking quality and physical appearance of seeds and in our studies we found that oils did not affect the germination of seeds. Schoonhoven (1978) also reported that there is no significant differences in the germination of treated and untreated seeds.

The use of oil is a method traditionally practised by the farmers of Sri Lanka to protect their seeds from bruchid attack. This is a cheap, convenient and effective method of protection in small farms and households if properly managed and the task before the extension workers is to educate the farmers effectively, so that it will increase the storability of pigeon pea.

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REFERENCES

Babu, T. R., Reddy, V. S. and Hussaini, S. H. (1989) Effect of edible and non edible oils on the development of the pulse beetle *Callosobruchus chinensis* L. on variability and yield of mungbean (*Vigna radiata* (L) Wilczek). *Trop. Scien.*, **29**: 215-220.

Chander, H. and Ahmed, S. M. (1986) Efficacy of oils from medicinal plants as protectants of greengram against the pulse beetle *Callosobruchus chinensis*. *Entomon.*, **11 (1)**: 21-28.

Hill, J. and Schoonhoven, A. V. (1981) Effectiveness of vegetable oil fraction in controlling the mexican bean beetle on stored beans. *J. Econ. Entomol.*, **74**: 478-479.

Khaire, V. M., Kachare, B. V and Mote, U. N. (1992) Efficacy of different vegetable oils as grain protectants against pulse beetle *Callosobruchus chinensis* L. in increasing storability of pigeon pea. *J. Stored Prod. Res.*, **28 (3)**: 153-156.

Mummigatti, S. G. and Ragunathan, A. N. (1977) Inhibition of multiplication of *Callosobruchus chinensis* L. by vegetable oils. *J. Food Sci. Tech.*, **14**: 184-185.

Pereira, J. (1983) The effectiveness of six vegetable oils as protectants of cowpeas bambara groundnuts against infestation by *Callosobruchus maculatus* (F.). *J. Stored Prod. Res.*, **19**: 57-62.

Raina, A. K. (1970) *Callosobruchus* species infesting stored pulses in India and comparative study of their biology. *Ind. J. Ent.*, **32**: 303-310.

Sangappa, H. K. (1977) Effectiveness of oils as surface protectants against the bruchid *Callosobruchus chinensis* L. infestation on redgram. *Mysore J. Agric. Sci.*, **11**: 391-397.

Schoonhoven, A. V. (1978) Use of vegetable oils to protect stored beans from bruchid attack. *J. Econ. Entomol.*, **76**: 254-256.

Singh, S. R., Luse, R. A., Leuschner, K. and Nangju, D. (1978) Groundnut oil treatment for the control of *Callosobruchus maculatus* during cowpea storage. *J. Stored Prod. Res.*, **14**. 77-80.

Strong, R. G., Partida, G. J. and Warner., D. N. (1968) Rearing stored product insects for laboratory studies, bean and cowpea weevil. *J. Econ. Ent.* **61**: 747–751.

Subasinghe, S. M. C. and Fellows, R. W. (1978) Recent trends in grain legume pest research in Sri Lanka. In *Pests of Grain Legumes*. Singh, S. R., Van Emdem, H. F. and Taylor, T. A. (eds), 37-41.

Van Huis, A. (1991) Biological methods of bruchid control in the tropics: A review. *Insect Sci. Applic.*, **12**: (1/2/3), 87-102.

Varma, B. K. and Pandey, G. P. (1978) Treatment of stored greengram seed with edible oils for protection from *Callosobruchus maculatus* (F.) Ind. J. Agric. Sci., **48**: 72-75.

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Electrophoretic Studies on Developmental profiles of Proteins in Haemolymph, Fat Body and Ovary of the Red Cotton Bug *Dysdercus koenigii* (Pyrrhocoridae: Heteroptera).

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Abstract: SDS–PAGE of haemolymph, fat body and ovary proteins of *Dysdercus koenigii* during the last larval instar and imaginal stages was conducted to find out the qualitative changes in protein patterns during larval–adult transformation. Comparison of electropherograms of the male and female revealed little difference. The number of proteins increased in all the three tissues till the third day of the 5th instar (synthetic phase) thereafter it decreased till the last day (consumption phase). Soon after eclosion, number of proteins in haemolymph decreased suggesting their role in cuticle formation. By contrast ovary and fat body show a sudden increase in number of proteins after the eclosion. Four protein bands are identified as major haemolymph proteins (MHP) in both the sexes during the said development stages.

Keywords: Protein patterns, SDS–PAGE, metamorphosis, Heteroptera, electrophoresis

INTRODUCTION

During metamorphosis of an insect, processes like destruction of certain larval tissues and rejuvenation and remoulding of various tissues into adult one are bound to take place involving synthesis and consumption of the macromolecules as well. Since proteins are the first biological factor(s) making their manifestation during development (Schmidt and Schwankl, 1975), studies on tissue specific proteins become morphogenetically of paramount significance.

Number of plasma proteins increase during successive stages of development (Kanost *et al.* 1990). Haemolymph of insect larvae contains several high molecular weight proteins like lipophorins (Shapiro *et al.* 1988) and arylphorins (Telfer *et al.* 1983) in addition to the storage proteins. All these proteins are synthezied in the fat body and released into the haemolymph (Kunkel and Lawler, 1974) to be incorporated later into various organs especially ovaries (Rohrkasten and Ferienze, 1985; Valle, 1993). Most

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of these studies were conducted in the holometabolous insects wherein stage specificity is well documented. On the other hand, the hemimetabolous insects, in which stage specificity is less distinct have received only scanty attention so far. The present study was undertaken on the premise to find out the qualitative changes in proteins of haemolymph, fat body and ovary of a hemimetabolous bug, *Dysdercus koenigii* during the larval–adult transformation. An attempt has also been made to unravel the possibility of the existence of any larval specific protein.

MATERIALS AND METHODS

Experimental Animals

D. koenigii was reared in the laboratory on soaked cotton seeds at 25–27°C under long day photoperiod (16h light; 8h dark) as described earlier (Venugopal *et al.* 1994).

Sample preparation

Haemolymph from precisely staged 5th instar larvae and imagoes of both sexes was collected separately in chilled calibrated microcapillary tubes and stored at −20°C until used. Fat body and ovary were dissected out in Insect Ringer Solution from insects already used for haemolymph collection, and washed thoroughly in the same. Wet weight of both the tissues were taken after blotting. 80 mg each of fat body and ovary was homogenised separately in hand driven glass microhomogeniser in 0.4 ml62.5 mM Tris–HCl buffer, pH 6.8 at 4°C, centrifuged at 10,000 rpm for 10 min at 4°C and the supernatant was stored at −20°C until used.

Polyacrylamide gel electrophoresis

SDS–PAGE (slab gel–130 × 130 × 3 mm of 10% separating and 5% stacking gel) was performed (Laemmli, 1970) using 62.5 mM Tris–Glycine buffer, pH 8.6 containing 0.1% SDS at a constant current of 10 mA for stacking and 20 mA for running gel for 3 hours. 2.5μl supernatant of haemolymph and 10μl supernatant of fat body and ovary each were mixed separately with 20μl of the sample buffer (62.5 mM Tris–HCl, pH 6.8; 1% SDS, 5% 2–mercaptoethanol, 10% Glycerol, 0.01% Glycerol, 0.01% Bromophenol Blue) in eppendorf tubes; boiled at 100°C for 3 min and cooled down to room temperature. 10μl each of these samples were loaded in the lanes of the gel. Following electrophoresis, gels were stained overnight in 0.25% Coomassie brilliant blue in a melange of 50% methanol and 10% acetic acid, and destained in a solution of 10% methanol + 10% acetic acid. Stained gels were stored in 7.5% acetic acid at 4°C.

RESULTS

SDS–PAGE under reducing and denaturing conditions was performed on male and female haemolymph, fat body and ovary of 5th instar larvae of various ages (0 day, 3rd day and 6th day) and 0 day imagoes in order to compare their protein patterns and also to find out the presence of any stage specific protein during these developmental stages.

Figure 1 illustrates the SDS–PAGE protein patterns of haemolymph, fat body and ovary of various ages of 5th instar larvae as well as 0 hr adults of both sexes. Comparison of these electropherograms reveal little differences between male and female.

Protein patterns of these tissues at the larval stage reveal the presence of some bands that disappear after molting; as well as some bands that appear after adult eclosion. These bands are numbered from their point of origin and are designated as minor and major bands according to their staining intensities. Patterns of different tissues are described as below:

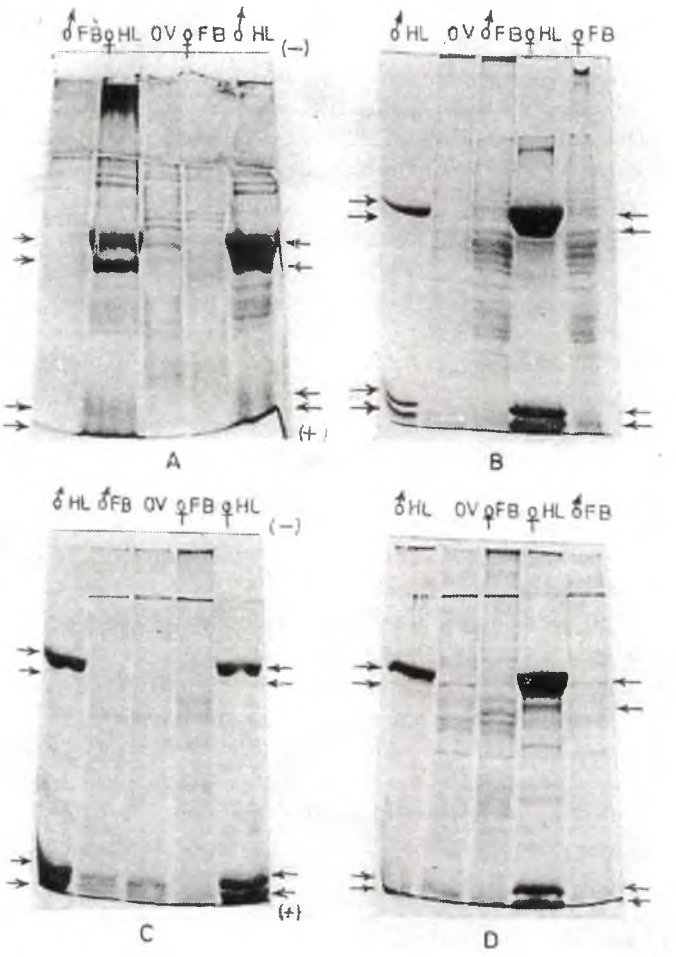

Fig. 1. Shows the SDS–PAGE patterns of fat body, haemolymph and ovary of *Dysdercus koenigii* during the last larval period and the imago phase A–0 day old 5th instar larva; B–3 day old 5th instar; C–6 day old 5th instar larva; D–0 day old adult. Arrow heads show the four major haemolymph proteins (MHP). Details regarding Rf values etc. are given in the text.

Haemolymph Protein Pattern (HPP)

The 0 day (0 hour) 5th instar female haemolymph resulted in 16 polypeptide bands of varying intensities. Of these, four bands (nos. 8, 9, 15, 16 with respective Rf values 0.5, 0.58, 0.92, 0.98) are thick and appear to be the subunits of major haemolymph

proteins of this stage, while rest of the protein zones are minor bands varying slightly in their intensities (Fig. 1A ♀ HL).

The 3 day (72 hr) old 5th instar female haemolymph exhibits 19 polypeptide bands of varying intensities (Fig. B ♀ HL). Out of these, band nos. 4, 5, 18 and 19 (with respective Rf values 0.5, 0.58, 0.92, 0.97) appear to constitute the subunits of the major haemolymph proteins. These four bands are the same proteins found in the haemolymph of 0 day as they show similar mobilities and hence same Rf values. Rest of the 15 protein zones are minor bands slightly differing in their intensities. The protein band numbers 1, 3, 6, 7, 12 and 13 (respective Rf values of 0.26, 0.31, 0.52, 0.55, 0.67, 0.69) present in 0 day perhaps disappear in the 3rd day haemolymph; and the 9 proteins bands (nos. 6, 8, 9, 11, 12, 13, 15, 16 and 17 with respective Rf values of 0.42, 0.53, 0.60, 0.71, 0.74, 0.76, 0.80, 0.84, 0.87) make their appearance a new on 3rd day (Fig. 1B, A ♀ HL).

SDS–PAGE of haemolymph from 6th day old female 5th instar resulted in only 14 protein bands, thus exhibiting a reduction in number of proteins from that 3rd day. But, the number of major subunits of haemolymph protein remains as four. These bands are numbered as 3, 4, 13 and 14 (respective Rf values of 0.50, 0.58, 0.92, 0.98) (Fig. 1C-♀-HL). Further comparison of 3rd and 6th day haemolymph electrophorogram reveals that protein band nos. 8, 10, 11, 12, 13 and 16 (respectively Rf values 0.53, 0.68, 0.71, 0.74, 0.76, 0.84) observed on 3rd day disappear on 6th day, whereas band no. 10 (Rf 0.82) makes its appearance on 6th day. Furthermore, male and female haemolymph do not show any significant difference in their protein patterns excepting on 3rd wherein female haemolymph shows 19 protein bands, and the male haemolymph could reveal only 9 protein zones. These 10 extra proteins of female haemolymph are band nos. 1, 2, 6, 8, 9, 10, 11, 13, 16, 17 (Rf values 0.42, 0.53, 0.60, 0.62, 0.67, 0.71, 0.76, 0.84, 0.87, 0.89 respectively) and are conspicuously absent in the male haemolymph.

After the metamorphosis *i.e.*, from 5th instar to imago (0 day old adult), there occurs a marked reduction in the number of proteins in female haemolymph (Fig. 1D and C) and the total number of protein bands at this stages are 11. It is observed that protein bands nos. 1, 8, 9, 10, 11 and 12 (respective Rf values 0.10, 0.82, 0.84, 0.89, 0.91, 0.94) of 6th day female instar disappear in the adult, whereas 0 day adult shows appearance of new protein band nos. 7, 8 and 9 (respective Rf values of 0.72, 0.74, 0.81).

Fat Body Protein Pattern (FBPP)

Figure 1 also illustrates the SDS–PAGE protein patterns of the fat body of both male and female 5th instar larvae of 0, 3 and 6 day as well as 0 day adult.

There are 17 protein bands in the 0 day 5th female instar electropherogram. Unlike the haemolymph proteins, the staining of different fat body proteins bands is very weak and they do not vary significantly among themselves. However, all these proteins exhibited as thin bands. As the development proceeds, on 3rd day (72 hr) the electropherogram exhibits a dramatic increase in the number of bands in comparison to that of the 0 day. Thus on 3rd day 25 proteins bands of varying intensities are revealed. Out of these, nineteen protein bands (nos. 2, 3, 4, 8, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25 with respective Rf values of 0.20, 0.23, 0.25, 0.45, 0.49,

0.51, 0.53, 0.58, 0.60, 0.63, 0.67, 0.69, 0.72, 0.75, 0.77, 0.79, 0.82, 0.85, 0.91 appear to be new proteins. Moreover, band nos. 1, 2, 3, 4, 5, 7, 8, 9, 13, 16 and 17 (respective Rf values 0.12, 0.15, 0.17, 0.21, 0.26, 0.29, 0.31, 0.33, 0.57, 0.65, 0.82) present in the 0 day fat body electropherogram have disappeared on the 3rd day. Further, on the 6th day of development the female fat body electropherogram reveals the presence of 16 protein bands exhibiting a reduction in the number of proteins. Thus, protein band nos. 1, 2, 3, 8, 10, 11, 16, 17, 18, 19, 20, 21, 22, 23 and 25 (respective Rf values of 0.16, 0.20, 0.23, 0.45, 0.49, 0.51, 0.63, 0.67, 0.69, 0.72, 0.75, 0.77, 0.79, 0.82, 0.91) present on 3rd day disappear here, whereas new protein bands (nos. 1, 2, 7, 10, 11, 13 with respective Rf values of 0.13, 0.21, 0.42, 0.53, 0.61, 0.71) appear (Fig. 1C and B-♀ FB).

As the fifth instar molts into adult, the fat body again shows an increase in its protein bands. SDS–PAGE resulted in the presence of 25 protein zones at this stage (Fig. 1D ♀ FB). Out of which band nos. 1, 3, 5, 6, 7, 8, 9, 10, 12, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24 (respective Rf values of 0.17, 0.21, 0.31, 0.36, 0.40, 0.42, 0.44, 0.48, 0.53, 0.57, 0.60, 0.63, 0.65, 0.69, 0.71, 0.73, 0.83, 0.85, 0.90) are new proteins. Further, protein band nos. 3, 7, 9, 10, 11, 12, 13, 14, 15 respective Rf values 0.34, 0.42, 0.53, 0.58, 0.61, 0.65, 0.71, 0.75, 0.80 of this instar (6 day old) disappear when it develops into imago (Fig. 1C and D).

Ovary Protein Pattern (OPP)

The SDS–PAGE protein patterns of ovary revealed significant differences among the larval and imaginal stages.

Fifth instar ovary on 0 day revealed 19 protein bands of varying intensities (Fig. 1A OV). Many of these proteins have similar mobilities to that of fat body and haemolymph proteins of the same age. Number of proteins in ovary increased to 23 on the 3rd day of 5th instar (Fig. 1B OV); and on this day protein band nos. 1, 3, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 19, 20 and 21 (respective Rf values of 0.15, 0.21, 0.52, 0.55, 0.59, 0.62, 0.65, 0.69, 0.72, 0.75, 0.79, 0.82, 0.85, 0.87, 0.90) appear as new proteins. While protein band nos. 1, 2, 3, 4, 5, 6, 7, 13, 14, 15 and 17 (respective Rf values of 0.18, 0.22, 0.23, 0.28, 0.31, 0.35, 0.39, 0.64, 0.68, 0.73, 0.76) observed on 10 day disappear on the 3rd day.

As the development proceeds, on 6th day, ovary shows the presence of 17 protein bands (Fig. 1C OV) revealing a marked reduction in the number of protein bands from that of the 3rd day. Thus, protein band nos. 1, 2, 5, 7, 9, 10, 11, 12, 13, 16, 17, 19, 20 and 23 (respective Rf values 0.15, 0.19, 0.35, 0.52, 0.59, 0.62, 0.65, 0.69, 0.70, 0.79, 0.85, 0.87, 0.95) present on the 3rd day disappear on 6th day. However, on 6th day, protein band nos. 1, 2, 6, 9, 10, 12, 13 and 16 (respective Rf values 0.10, 0.15, 0.42, 0.62, 0.67, 0.75, 0.80, 0.91) make their appearance as new polypeptides as compared to 3rd day. Furthermore, the SDS–PAGE of imago ovary resolved into 25 protein bands (Fig. 1D OV) again showing an increase in proteins. 0 day adult ovary protein band nos. 1, 2, 3, 4, 6, 9, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 with respective Rf values of 0.12, 0.15, 0.19, 0.21, 0.26, 0.37, 0.52, 0.56, 0.61, 0.65, 0.68, 0.73, 0.79, 0.81, 0.84, 0.89 as new proteins in comparison to that of 6th day. Whereas protein zones 1, 2, 6, 10, 11, 12, 13, 14 and 15 with respective Rf values of 0.10, 0.15, 0.42, 0.67, 0.71, 0.75, 0.80, 0.85, 0.89 present on the 6th day of instar development disappear when it molts into the imago.

DISCUSSION

Results of the present SDS–PAGE investigation reveal that number of proteins in haemolymph, fat body and ovary increase during the first half of the fifth larval period *i.e.*, from 0 to 3rd day and then decline in the second half *i.e.*, from 3rd to 6th day. Such qualitative changing profiles of proteins observed during larval–adult transformation confirms our earlier findings wherein the same stages of the same insect had shown quantitative changes in proteins (Venugopal *et al.* 1994). Based on this finding we had assigned the last larval stage into 'synthetic and utilization phase'.

Electropherograms further reveal the presence of four major haemolymph polypeptide bands (Cf Fig. arrows). It is speculated that these bands in *D. koenigii* might arise in the last larval stage as true for holometabolous insects (Chrysanthis *et al.* 1981; Roberts and Brook, 1981; Levenbook, 1985). Nevertheless, the aforesaid proteins cannot be correlated with the larval proteins of holometabolus insects as they are also detectable in the electropherograms of imago (Cf. Fig. 1D). On the other hand, one can attribute a different function(s) to these proteins than the storage proteins. It is, however, clear from the electropherograms that during larval–adult transformation, some proteins disappear while some new one appear, especially in the synthetic phase (Venugopal *et al.* 1994). It is also observed that a few bands appear during the consumption phase. This phenomenon of appearance and disappearance of proteins in a phased manner implicate a genetic control mechanism (Rockstein, 1978). Notwithstanding, the appearance of some polypeptides of haemolymph in ovary could be the result of sequestration of the former (Levenbook, 1985). Since the observed differences between proteins of both the sexes are slight at the larval stage, it is presumed that the proteins contributing to the initial development of testes are not very different from those used for building up the ovaries (Schmidt and Wirth, 1974).

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REFERENCES

Chrysanthis G., Marmaras, V. J. and Chrystodoulous, C. (1981) Major haemolymph proteins in *Ceratitis capitata*. Wilham Roux's Archieves. **190**: 33–39.

Kanost, M. R, Kawooga, J. K., Law, J. H., Ryan, R. O., Heusden, M. C. and Zeigler, R. (1990) Insect haemolymph proteins. In Adv. in Insect Physiol., Academic Press. **22**: 299–397.

Kunkel J. G. and Lawler, D. M. (1974) Larval specific serum proteins in the order *Dictyoptera–I*. Immunological actericharzation in larval *Blattella germanica* and cross reaction throughout the order. Comp. Biochem. Physiol. **B47**: 697–710.

Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature. **227**: 680–685.

Levenbook, L. (1985) Insect storage proteins. *In Comprehensive Insect Physiology, Biochemistry and Pharmacology* (Edited by Kerkut G. A. and L. I. Gilbert), Academic Press.

Roberts D. B. and Brook, H. W. (1989) The major serum proteins of Dipkteran larvae. Experientia. **37**: 103–212.

Rockstein M. (1978) Biochemistry of Insects. Academic Press, New York. 146.

Rohrkasten A. and Ferenze, H. J. (1985) *In vitro* study of selective endocytosis of vitellogenin by locust oocytes. *Roux's Arch. Dev. Biol.* **194**: 411–416.

Schmidt G. H. and Schwankl, W. (1975) Changes in haemolymph proteins during the metamorphosis of both sexes and castes of polygynous *Formica rufa*. *Comp. Biochem. Physiol.*, **B52**: 365–380.

Schmidt G. H. and Wirth, B. (1974) Disk–electrophorestische Frakionierung der Hamolymphe–Proteine Wahrend der metamorphose verschie denere Kasten Und Geschle Chter Vow Formica Pratensin. *J. Insect. Physiol.* **20**: 1421–1466.

Shapiro J. P., Wells, M. A., Law, J. H. (1988) Lipid transport in insects. *Ann. Rev. Entomol.* **33**: 297.

Telfer W. H. and Keim, P. S., Law, J. H. (1983) Arylphorin, a new protein from *Hyalophora cecropia*: Comparison with calliphorin and manducin. *Insect Biochem.* **13**: 601.

Valle D. (1993) Vitellogenesis in insects and other groups – A review. *Mem. Inst. Oswaldo. Cruz.* **88**: 1–26.

Venugopal K. J., Dinesh Kumar and Singh, S. A. (1994) Developmental studies on proteins from haemolymph, fat body and ovary of a phytophagous bug, *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae). *Biochem. Arch.* **10(4)**: 297–303.

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Studies on *Sphegigaster* Spinola (Hymenoptera: Chalcidoidea: Pteromalidae) from India

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Abstract: Two new species viz. *Sphegigaster reticulata* and *Sphegigaster anamudiensis* are described from India. Besides *S. brunneicornis* (Ferriere) is reported for the first time from India and *S. stepicola* Boucek from Kerala. A key to the Indian species of *Sphegigaster* is also provided.

Keywords: *Sphegigaster*, Chalcidoidea, Pteromalidae, New species, New records.

INTRODUCTION

The genus *Sphegigaster* Spinola contains several species reported from all the continents. From India so far only one species viz. *S. stepicola* Boucek has been reported by Boucek *et al* (1979). Boucek (1988) synonymised *Paratrigonogastra* Girault and *Basilewskyella* Risbec with *Sphegigaster* and listed *Trigonogastra* Ashmead as a junior synonym of *Sphegigaster*.

In this paper two new species, *Sphegigaster anamudiensis* and *Sphegigaster reticulata* are described from Kerala and one species, *Sphegigaster brunneicornis* (Ferriere) is recorded for the first time from India. *Sphegigaster stepicola* Boucek is also recorded for the first time from Kerala.

Key to the Indian species of Sphegigaster

1. Antennae short (Fig. 7), all funicle segments transverse; F1 distinctly shorter than pedicel; gaster (Fig. 6) short, oval, length 1.7x width (without petiole); scape brownish black; femora darker, hind femur brown; smaller species; length 1.5–2 mm ..... *stepicola* Boucek.

   Antennae slender, not short, all funicle segments not transverse; F1 longer or very slightly shorter than pedicel; gaster elongatedly ovate, length 2.2–2.3x width

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(without petiole); scape yellow or testaceous; femora lighter, hind femur testaceous; larger species; length 2.5–3.2 mm ..... 2.

2. Combined length of pedicel plus flagellum 1.8x eye length; POL 1.6x OOL; petiole moderately reticulate even on sides; hind margin of T1 straight (Fig. 13); antennae (Fig. 10) uniformly yellow ..... *reticulata* sp. nov.
   Combined length of pedicel plus flagellum 2-2. 4x eye length; POL almost equal to OOL, only very slightly longer; petiole finely to moderately reticulate, but smooth on sides; antennae not uniformly yellow ..... 3.

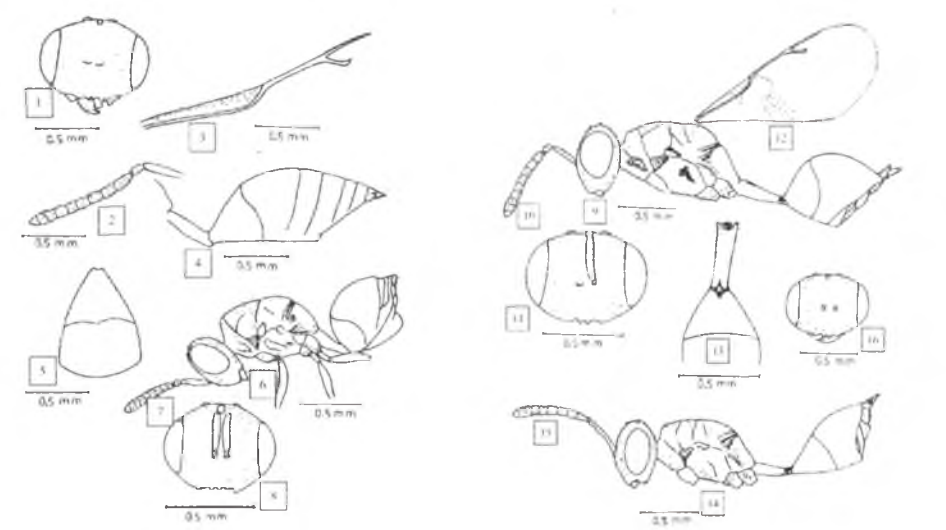
3. Antennae (Fig. 15) inserted middle of face; pedicel short, not much longer than broad; pedicel plus flagellum length 2 × that of eye; F1 not much slender; gaster (Fig. 14) without petiole not longer than thorax; antennae with scape, pedicel and ring joints yellow; legs except coxae yellow; length 2-2.5 mm ... *brunneicornis* (Ferriere).
   Antennae (Fig. 2) inserted little below middle of face; pedicel long, 1.8x longer than broad; pedicel plus flagellum length 2.4x that of eye; F1 slender and longer; gaster (Fig. 4) without petiole distinctly longer than thorax; antennae with scape testaceous; pedicel and ring joints dark brown as on flagellum; legs except coxae testaceous; length 3.2 mm ..... *anamudiensis* sp. nov.

***Sphegigaster anamudiensis* sp. nov. (Figs. 1–5)**

Female: Length 3.2 mm. Body bluish green with slight golden reflection on head and thorax; gaster including petiole and lateral parts of thorax dark blue, almost black; scape testaceous, remainder of antennae dark brown. Legs with coxae concolorous with thorax, remainder testaceous with tips of tarsi brown. Tegulae brown; wings hyaline, veins brown.

Head: In dorsal view width 1.2x that of thorax and 2x length; temple length 0.7x that of eye; POL almost equal to OOL; in front view (Fig. 1) head width 1.4x height; eyes separated by 1.4 × their height with inner orbits straight; malar space length 0.47x that of eye. Head moderately reticulate, finer on clypeus and adjacent areas. Antennae (Fig. 2) inserted below middle of face; scape length 0.76x that of eye, reaching median ocellus; pedicel plus flagellum length 1.1 × head width.

Thorax: Length 1.6x width; pronotal collar length 0.2x that of mesoscutum (median length), sharply margined anteriorly, moderately reticulate on anterior half, smooth on posterior half. Mesoscutum width 2x length, moderately reticulate. Scutellum moderately convex, length equal to width, similarly reticulate as on mesoscutum. Dorsellum weakly reticulate. Propodeum width 2.6x median length; median area similarly sculptured as on scutellum, lateral parts finely reticulate; median carina not indicated; median area slightly elevated: plicae indicated anteriorly, area adjacent to them depressed; spiracles elongatedly ovate; post–spiracular sulcus deep; callus with sparse pubescence. Mesopleuron moderately reticulate, finer on anterior parts; prepectus broad, finely reticulate. Metapleuron finely reticulate. Forewing (Fig. 3) length 2.3x width; costal cell hairy on upper half only; basal cell setate; speculum open below; basal part of wing almost bare. Relative lengths of smv, mv, pmv, and stv as 39:25:18:9. Legs slender.



Figs. 1–5. *Sphegigaster anamudiensis* sp. nov. Female 1. Head in front veiw. 2. Antenna 3. Forewing venation 4. Gaster with petiole in profile 5. Gaster in dorsal view – T1 & T2.
Figs. 6–8. *Sphegigaster stepicola* Boucek. Female 6. Body of profile 7. Antenna 8. Head in front view.
Figs. 9–13. *Sphegigaster reticulata* sp. nov. Female 9. Body in profile 10. Antenna 11. Head in front view 12. Forewing 13. Gaster dorsal view with petiole and T1.
Figs. 14-16. *Sphegigaster brunneicornis* (Ferriere). Female 14. Body in profile 15. Antenna 16. Head in front view.

Gaster: (Figs. 4 and 5) Length 1.2x that of thorax in profile and length 2.2x width (without petiole) in dorsal view; petiole length 0.3x that of gaster in profile.

Male: Unknown.

Holotype ♀ , India: Kerala, Eravikulam National Park (Anamudi) 28.02.1995, Coll. C. Radhakrishnan and Party. (Deposited in WGFRS ZSI Calicut).

Remarks: This species closely resembles *S. brunneicornis* (Ferriere) but differs from it as follows: (1) antennae with pedicel and ring joints dark brown as on flagellum, inserted little below middle of face; pedicel not short, almost 2x as long as broad (in *brunneicornis* pedicel and ring joints clear yellow, antennae inserted middle of face; pedicel short not much longer than broad) (2) gaster without petiole longer than thorax (in *brunneicornis* gaster not longer than thorax (3) forewing with veins not very pale, pmv much shorter than mv; stv only about 0.3x mv (in *brunneicornis* veins very pale, pmv not much shorter than mv, and stv about 0.7x mv) (4) legs except coxae testaceous, mandibles dark brown, head moderately reticulate and larger species, length 3.2 mm (in *brunneicornis* legs except coxae clear yellow, mandibles yellowish brown and head very finely reticulate smaller species length 2-2.5 mm).

***Sphegigaster reticulata* sp. nov. (Figs. 9–13)**

Female: Length 2.7 mm. Head, thorax and petiole bluish black; gaster black; eyes dark red; antennae yellow; all coxae concolorous with thorax, remainder of legs brownish yellow with tips of tarsi brown; Tegulae testaceous; veins pale yellow.

Head: (Figs. 9 and 11) In dorsal view width 2x length and in front view width 1.3x height; uniformly and finely reticulate; temples converging strongly, length almost half of eye length; POL 1.6x OOL; eye length 1.6x width (in profile), separated by 1.2x their length; malar space length half of eye length. Antennae (Fig. 10) inserted above lower margin of eyes; scape hardly reaching median ocellus, length 0.7x that of eye; combined length of pedicel and flagellum 1.8 × eye length; F1 very slightly shorter than pedicel, funicle segments longer than wide, except last two almost as long as wide; club about as long as 2.5 preceeding segments combined.

Thorax: (Fig. 9) Pronotal collar nearly rectangular, uniformly recticulate except on a narrow smooth strip posteriorly. Mesoscutum and scutellum moderately reticulate; notaular grooves appears complete when looking at certain angles. Mesoscutum width about twice length. Scutellum slightly convex, almost as long as wide. Propodeum width 2.2x length, similarly sculptured as on scutellum, except on callus finely reticulate; median carina not indicated; spiracles small and oval, prepectus broad without any fovea, finely reticulate. Metapleuron finely but distinctly reticulate. Forewing (Fig. 12) with basal vein setate; speculum open below. Relative lengths of smv, mv, pmv and stv as 33.5:20:14.5:7.

Gaster: (Figs. 9 and 13) Elongate, ovate, length 2.3 × width; petiole moderately reticulate even on sides, length 3.6x width; T1 occupying almost one third length of gaster; hind margin straight; T2 occupying more than half of gaster.

Male: Unknown.

Holotype, ♀ India: Kerala, Parambikulam, 6.05.1989. Coll. P. M. Sureshan. (Deposited in WGFRS, ZSI, Calicut).

Remarks: This species closely resembles *S. intersita* Graham, but differs from it as follows: (1) antennae with scape and pedicel yellow (in *intersita* antennal scape and pedicel black with a metallic tinge) (2) POL 1.6 × OOL (in *intersita* POL only 1.1 to 1.2x OOL) (3) pronotum with lateral angles of collar without teeth (in *intersita* lateral angels of collar forming blunt teeth and three other rather weak teeth on front edge of collar) (4) mesoscutum, scutellum and propodeum moderately reticulate (in *intersita* mesoscutum, scutellum and propodeum finely reticulate) (5) mv 2.9x stv and 1.4 × that of pmv (in *intersita* mv only 2x stv and only very slightly (1.1x longer than pmv)) (6) petiole length 3.2x width, gaster length 2.3x width (in *intersita* petiole length 2.7–3x width and gaster length 2.7x–2.9x width).

***Sphegigaster stepicola* Boucek (Figs. 6–8)**

Sphegigaster stepicola Boucek, 1965, *Acta Faun. ent. Mus. Nat. pragae*: **11**: 12–14.
Acroclisis melanagromyzae Mani, 1971, *J. nat. Hist:* **5**: 591–593.

Remarks: Till the recognition of *Acroclisis melanagromyzae* as *Sphegigaster stepicola* by Boucek *et al* (1979) this species was known only from the Mediterranean subregion. Boucek *et al* (1979) also recorded this species from Delhi and Bihar. This forms the first record of the species from Kerala. The main diagnostic features of the species are as follows: smaller species, length varies between 1.5-2 mm; body slender, shiny black; legs relatively dark; femora mainly so; tibiae more or less infuscate; antennae (Fig. 7) with all funicle segments transverse; F1 obviously shorter than pedicel;

gaster (Fig. 6) oval, shorter than thorax; petiole length nearly 3x width; hind margin of T1 with its middle portion truncate or virtually so.

Material examined: 5 females, Kerala, Kayamkulam, 21.02.1989; 4 females 1 male, Anakatty (Palghat), 12.12.1987; 2 females, Ochira, 26.02.1989; 3 females, Ernakulam, 9.02.1989; 1 female, 1 male, Nedumpoyil (Cannore), 24.02.1988; 3 females, Kasargod, 27.02.1988; 1 female, 1 male, Shertallai, 27.02.1989; 2 females, Peravoor (Cannore), 25.02.1988; 1 female, Vayalar, 27.02.1989; 2 females, Attingal, 23.02.1988; 1 female, Kazhakuttom, 25.02.1989; 2 females, Katakkada (Trivandrum), 24.02.1989; 1 female, Kappil (Trivandrum), 26.02.1989; 2 females, Kottiyam (Quilon), 23.02.1989; 1 female, Manjeswaram, 27.02.1988; 4 females, Calicut University Campus, 23.02.1989, (Coll. P. M. Sureshan); 2 females, Calicut University Campus, 11.1986 and 04.1987, Coll. T. C. Narendran and Party.

Distribution: India (Kerala, Delhi, Bihar, U. P.), Austria, Czechoslovakia, Moldavian SSR and Algeria.

Biology: Reported from India as a parasite of *Melanagromyza* and *Phytomyza* (Agromyzidae: Diptera) mining the leaves of *Helianthus* and of *Vigna catjang* (Mani, 1971). Reared in Austria from *Phytomyza albiceps* Mg. on *Cirsium arvense* (L.) (Boucek, 1965).

***Sphegigaster brunneicornis* (Ferriere) (Fig. 14–16)**

Trigonogastra brunneicornis Ferriere, 1930. *Bull. ent. Res.* **21**: 356–357.

Remarks: Boucek *et al* (1979) designated the lectotype of this species and put *Trigonogastra brunneicornis* Ferriere under *Sphegigaster* as new combination. This forms the first record of the species from India. The diagnostic features of the species are as follows: Body dark green, almost black, especially on head; gaster dark green; antennae (Fig. 15) with scape, pedicel and ring joints clear yellow, flagellum reddish brown; F1 longer than pedicel, narrowed basally; all funicle segments longer than wide except F6 subquadrate; club almost as long as two preceeding segments combined; pronotum distinctly carinate; propodeum without indication of median carina; gaster (Fig. 14) without petiole not longer than thorax; body length varies between 2-2.5 mm.

Material examined: 4 females, Kerala, Calicut (West hill), 25.05.1987; 11 females, Kalkandi (Agali), 13.12.1987; 2 females, Agali, 13.12.1987; 2 females, Kottiyam (Quilon) 23.02.1989; 1 female, Silent Valley, 30.12.1980; 1 female, Puzhamudi, 23.02.1988; 2 females, Thariyod, 22.02.1988; 1 female, Manantody, 2.02.1988, (Wynad); 1 female, Nedumpoil, 24.02.1988; 1 female, Aralam farm, 25.02.1988; (Cannore); 2 females, Kayamkulam, 19.02.1989; 1 female, Neendakara, 22.02.1989; 1 female, Manjeswaram, 27.02.1988; 3 females, Malampuzha, 11.12.1987 and 16.01.1986; 3 females, Shertallai, 27.02.1989; 2 females, Anakatty (Palghat), 12.12.1987; 1 female, Peechi (Trichur), 5.02.1989; 1 female, Chungathara (Malappuram), 24.02.1989; 1 female, Calicut University Campus, 3.06.1988; (Coll. P. M. Sureshan); 2 females, Thekkadi, 05.1986, Coll. T. C. Narendran and Party.

Distribution: India (Kerala), Sri Lanka.

Biology: Reported from Sri Lanka as a parasite on pupae of *Agromyza* sp. mining stems of *Hibiscus esculentus* (Ferriere, 1930) and *Ophiomyia phaseoli* (Tryon) on beans. (Boucek *et al*, 1979).

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REFERENCES

Boucek, Z. (1965) A review of the Chalcidoid fauna of the Moldavian SSR with descriptions of new species (Hymenoptera). *Acta. Faun. ent. Mus. Natn. Pragae.* **11**: 5–37.

Boucek, Z. (1988) Australasian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of fourteen families, with a reclassification of species. CAB *International, Wallingford.* 1–831.

Boucek, Z., Subba Rao, B. R. and Farooqui, S. I. (1979) A preliminary review of Pteromalidae (Hymenoptera) of India and adjacent countries. *Oriental Ins.* **12**: 433-467.

Ferriere, C. (1930) Notes on Asiatic Chalcidoidea. *Bull. ent. Res.* **21**: 353–360.

Mani, M. S. (1971) Some Chalcidoid parasites (Hymenoptera) of leaf mining Agromyzidae (Diptera). *J. nat. Hist.* **5**: 591–598.

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Effects of Human Insulin on the Intermediary metabolism of *Teleogryllus mitratus*

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Abstract: Insulin has various well established roles in vertebrates, but the effects if any, of this hormone in invertebrates are less understood, especially in insects. Vertebrate (human) insulin (5 ng and 10 ng/insect) was injected in the abdominal region of 48 h old adult crickets, *Teleogryllus mitratus*. The possible effect of this hormone on the intermediary metabolism was studied. For this, the concentration of certain biochemical constituents like protein, glycogen and the activities of the enzymes viz; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and trehalase were evaluated, 48, 72, 96, 120 and 192 h after the administration of the hormone. Fat body, ovary and haemolymph were taken from the experimental and control insects for biochemical studies. A time dependent increase in the enzyme activities and biochemical constituents was observed in both experimental and control insects. However, the increase was more pronounced in hormone treated insects.

Keywords: Alanine aminotransferase (ALT), Asparate aminotransferase (AST), Insulin, *Teleogryllus mitratus*.

INTRODUCTION

During the course of evolution, invertebrates have undergone several significant changes in their body structure and functions. Some of the peptides found in insects are unique, while some others show immunochemical similarity with vertebrate peptides. The peptide hormones are the best examples. The advancements of analytical techniques like RIA and immunohistochemistry are helpful in identifying these peptides in vertebrates. The peptide hormones are detected in the neuroendocrine system and also in the gastrointestinal tissues.

In vertebrates, the function of these peptides are well documented. But in invertebrates, very little is known about the functions of these peptides. The major problem was to elucidate their chemical structure. There are several recent references which show the presence of insulin like compounds/peptides in a large number of insects (Yu *et al* 1993, Pratt *et al.*, 1990). It is difficult to identify which peptide is present and

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active in which insect and at what stage? So, more informations about isolation, characterization and molecular sequencing are needed. The procedures employing RIA can he used to characterize the peptides of the insects also (Gilbert and Kerkut 1985). By using this technique, insulin or closely related peptides are identified in insects like *Apis mellifera* (Ishay *et al.*, (1976). They have identified insulin-like peptides from adult insect gut and larval mid gut. Yui *et al.* (1980) reported that the brain, corpora cardiaca and corpora allata of *Bombyx mori* contain insulin like peptides. In hoverfly, *Eristatis aeneus*, insulin like peptides are reported in the brain (El-Salhy *et al.*, 1980). Presence of insulin or insulin-like peptides have been reported in the haemolymph, brain corpora cardiaca and corpora allata of *Manduca sexta* (Kramer *et al.* 1986, 1987).

Some of the earlier workers were able to point out various aspects of the functions of these peptides. Their regulatory effects in the intermediary metabolism are also well documented in vertebrates. However, the physiological effects of these hormones in insects are less explored. In the light of the above reports, it appears that the peptides are of importance in the metabolism of lower animals and therefore it was thought worth while to study the possible effects of one of these (Insulin) on the intermediary metabolism of *Teleogryllus mitratus*.

MATERIALS AND METHODS

Nymphs were collected and kept in separate containers for obtaining newly moulted adults. Adult (48 h old) were injected with two different doses (5 ng and 10 ng/insect) of insulin (Novlin Lente Human Insulin Zinc suspension, Recombinant DNA origin from Novo Nordisk A/S, DK-2880 Bags-Vaerd, Denmark). The control insects received saline injections. Haemolymph, fat body and ovary from the experimental and control insects were collected 48 h after hormone administration. The activities of the enzymes AST, ALT and trehalase and the concentration of protein and glycogen were measured. The enzyme assay of AST and ALT was done according to the procedures of Reitman and Franknel (1957). Protein content was determined by the method of Lowry *et al.*, (1951) and glycogen was estimated according to the procedure of Seifter *et al.*, (1950). Estimation of trehalase was done spectrophotometrically based on the digestion of substrate trehalose and the method followed was that of Neolling and Bernfield (1948) as modified by Ishaaya and Swirski (1970). Student t-test was employed for statistical analysis of the data.

RESULTS

Results presented in Table 1 show, a dose and time dependent increase in the level of protein in the fat body. This increase was most prominent in 10 ng insulin treated insects. The activities of ALT and AST increased due to the administration of 5 and 10 ng of insulin when compared to the controls and once again the response obtained was dose and time dependent. The increase in the enzyme activity of trehalase was only seen with 10 ng treatment, while administration of 5 ng of insulin did not have any effect. The glycogen content in 5 ng treated insects was more or less the same when compared to the controls, but 10 ng treated insects revealed a significant increase (Table 1).

Table 1. Effect of Insulin on the Fat Body of *T. mitratus* (mean ± SD of 5 samples)

Hours after treatment	Protein			AST			ALT			Glycogen			Trehalase		
	C	5ng	10ng	C	5ng	10ng	C	5ng	10ng	C	5ng	10ng	C	5ng	10ng
48	7.13±	7.59±	12.89**±	2.78±	3.30±	5.18±	2.69±	3.25±	4.40±	3.35±	3.41±	4.23±	157±	164.33±	195.66±
	1.43	0.95	3.81	0.38	0.50	0.42	0.28	0.37	0.66	0.10	0.35	0.16	3.57	5.00	5.85
72	10.02±	12.57±	13.81±	3.65±	4.35±	5.63±	4.39±	4.44±	5.96±	4.01±	4.02±	4.67±	186.83±	190.33±	223.83**±
	1.31	2.00	4.20	0.36	0.50	0.53	0.19	0.14	0.83	0.19	0.22	0.29	4.26	3.26	5.84
96	19.71±	13.64±	16.12±	4.17±	6.20**±	6.71**±	4.92±	6.82**±	9.66**±	4.09±	4.23±	4.96±	209.33±	225.50±	281.66**±
	1.89	4.12	3.12	0.18	0.42	0.52	0.21	0.28	0.24	0.16	0.07	0.41	7.89	4.18	10.32
120	17.26±	18.08±	21.21±	4.80±	7.66**±	8.10**±	5.33±	10.61**±	11.29**±	4.60±	5.13±	5.93**±	392.16±	416.83±	612.16**±
	4.00	4.48	6.09	0.18	0.38	0.30	0.19	0.37	0.26	0.22	0.39	0.26	4.35	7.40	6.59
192	18.73±	23.19±	24.96±	1.96±	8.95**±	11.18**±	5.93±	11.55**±	11.82**±	4.896±	5.67±	6.51**±	454.86±	455.33±	799.66**±
	4.17	6.46	5.85	0.09	0.24	0.65	0.09	0.31	0.44	0.18	0.16	0.13	6.96	6.43	9.15

*<0.05 Protein μg/mg Trehalase μg glucose liberated/mg tissue

** <0.01 AST IU/mg wet wt. Glycogen μg/mg

ALT IU/mg wet wt.

Table 2. Effect of Insulin on the Ovary of *T. mitratus* (mean ± SD of 5 samples)

Hours after treatment	Protein			AST			ALT			Glycogen			Trehalase		
	C	5ng	10ng	C	5ng	10ng	C	5ng	10ng	C	5ng	10ng	C	5ng	10ng
48	8.88±	9.64±	10.63±	3.20±	3.81±	5.08±	6.46±	6.13±	6.40±	3.45±	3.49±	4.08±	321.33	399.16±	195.66±
	1.07	2.54	2.32	0.25	0.38b	0.42b	0.20	0.40	0.38	0.26	0.30	0.29	8.71	5.11	4.02
72	10.05±	10.29±	11.26±	4.03±	4.50±	6.23±	7.11±	6.65±	7.55±	3.97±	4.03±	4.94±	348.50±	350.16±	415.83**±
	1.49	1.83	2.37	0.37	0.54	0.45	0.36	0.68	0.52	0.23	0.26	6.59	6.24	7.02	5.84
96	12.04±	11.74±	12.93±	5.71±	6.26±	7.91±	7.58±	8.16±	8.50±	4.73±	5.37±	6.59±	383.50±	437.50**±	474.33**±
	2.59	1.79	7.87	0.47	0.33	0.33	0.34	0.25	0.31	0.14	0.54	0.32	9.07	6.18	9.64
120	13.07±	15.63±	16.48±	6.38±	7.23±	8.63**±	8.45±	8.50±	8.93±	8.79±	7.75±	8.53±	720.00±	872.50**±	852.00**±
	2.40	3.83	3.44	0.34	0.41	0.41b	0.32	0.40	0.13	0.28	0.17	0.19	60.00	39.86	44.26
192	13.68±	15.50±	15.93±	7.98±	8.18±	9.41**±	9.10±	9. 11±	9.73±	6.30±	9.70**±	10.17**±	960.00±	999.00±	114.84**±
	2.83	3.30	4.07	0.39	0.44	0.30	0.43	0.21	0.19	0.22	0.11	0.17	27.33	48.45	9.15

*<0.05 Protein μg/mg Trehalase μg glucose liberated/mg tissue

**<0.01 AST 1U/mg wet wt. Glycogen μg/mg

ALT 1U/mg wet wt.

Table 3. Effect of Insulin on the Hemolymph of *T. mitratus* (mean ± SD of 5 samples)

Hours after treatment	Protein			AST			ALT			Trehalase		
	C	5ng	10ng	C	5ng	10ng	C	5ng	10ng	C	5ng	10ng
48	2.11±	2.24±	2.99±	2.16±	2.95±	3.23±	2.75±	3.30±	3.43±	120.33±	128.50±	24.00**±
	0.44	0.36	0.41	0.53	0.33	0.48	0.22	0.34	0.39	6.25	2.51	6.98
72	3.54±	3.51±	4.19±	1.56±	2.78±	4.21±	3.06±	3.85±	5.03±	137.83±	148.00**±	270.00**±
	1.52	1.48	0.52	0.32	0.29	0.51	0.19	0.16	0.52	3.12	1.89	5.51
96	4.53±	4.56±	5.40±	3.10±	5.00±	5.63*±	3.36±	3.40±	5.00**±	213.16±	231.66	304.33**±
	1.42	1.33	1.29	0.28	0.52	0.67	0.30	0.31	0.67	18.56	4.50	6.62
120	7.04±	7.27±	9.46±	3.73±	5.56±	6.33**±	4.13±	4.45±	6.13*±	311.50±	328.66**±	355.50**±
	2.03	2.14	1.53	0.63	0.56	0.42	0.25	0.25	0.33	8.31	2.73	10.74
192	9.07±	9.32±	11.67±	5.51±	7.10±	7.88*±	4.96±	5.45 *pm*	6.98*±	366.00±	338.00±	426.00**±
	2.67	2.83	2.23	0.48	0.30	0.46	0.31	0.25	0.25	5.96	9.26	11.09

*<0.05 Protein μg/ml Trehalase μg glucose liberated/μl

** <0.01 AST 1U/ml

ALT 1U/ml

Insulin treatment did not show significant difference in the protein levels when compared with controls (Table 2). The activity of AST showed an increase in insulin treated insects and this increase was more prominent in 10 ng insulin treated insects as compared to 5 ng treated group. However it is interesting to note that the activity of ALT was more or less the same in experimental as well as control insects. Glycogen content of the tissue was altered when treatment was either carried out for 120 or 192 h with both the dosages. The activity of trehalase was significantly high in 10 ng insulin treated insects, while 5 ng insulin treated insects also showed lower level of effect at 72 h (Table 2).

Results presented in Table 3 show the effect of insulin treatment on haemolymph. Lower dosage of insulin did not show significant change in the protein and glycogen content or activities of ALT and AST enzymes. However, administration of 10 ng insulin significantly elevated the levels of protein and enzyme activities (Table 3).

DISCUSSION

In *Teleogryllus*, the fat body shows an increase in the protein content after insulin treatment and this increase is dose and time dependent. Similarly the increase is also seen in the protein content of ovary and haemolymph. The present study suggests that insulin treatment stimulates protein synthesis in this insect. The transaminase activities are increased after insulin treatment in fat body, ovary as well as haemolymph. The increase in transaminase activity indicates an increase in gluconeogenesis by increasing the level of aspartic acid or alanine. These amino acids are glucogenic and are directed to the TCA cycle by transamination to produce glucose molecules (Lehninger 1975).

Increase in trehalase activity might be reponsible for the hydrolysis of trehalose into glucose, which is utilized for the synthesis of glycogen. Further more insulin treatment shows stimulatory effect on glycogen of the fat body and ovary which is again dose-dependent. Insulin stimulates, directly or indirectly, organic metabolism and facilitates glucose transport across the membrane. This will stimulate the uptake of glucose by cells and promote glycogen and fat synthesis (Lehninger, 1975).

Stanchfield and Yager (1979) reported that insulin maintained high levels of protein synthesis in amphibian haemocytes. The administration of insulin rapidly augments the amino acid incorporation into proteins in most of the tissues especially in the liver. The *in vitro* experiments in rat diaphragm showed the same effect (Smith *et al.*, 1983). These effects reflect a major stimulus to the biosynthesis of proteins, an inhibitor of proteolysis in control and experimental animals.

Vertebrate insulin is known to have various types of physiological actions. In planarian, *Polyeclis nigra*, the glucose uptake is stimulated by insulin (Csaba and Kadar, 1978), while in protozoan, *Tetrahymena pyriforms*, glucose uptake as well as cAMP levels are stimulated after insulin treatment (Csaba and Lantos, 1976). It also stimulates the uptake of glucose in *Vespa orientalis* (Ishay, 1975). The general effect of vertebrate insulin includes growth stimulation, lipid mobilization, sugar uptake and cellular internalization. A note worthy effect of vertebrate insulin on insect is its ability to stimulate growth. In *Drosophila*, the growth of imaginal disks and several cell lines in culture were reported (Davis and Shearn, 1977). This peptide is used to replace the serum embryonic cell cultures *in vitro* of *Drosophilia* (Mosna, 1981). The principal role of serum in both mammalian and insect cell culture media is to supply various

nutrients and hormones. Apparently the *Drosophila* cells have an absolute requirement for insulin.

The exact function of vertebrate (human) insulin in the invertebrate system, especially in insects, is not fully known. The present work in *Teleogryllus* suggests that human insulin has considerable influence in stimulating the metabolic activities in *Teleogryllus mitratus* by promoting proteogenesis, gluconeogenesis, glycogenesis and also other anabolic reactions.

REFERENCE

Csaba, G. and Lantos, T. (1976) Effects of insulin on the glucose uptake of protozoa *Experientia* **31**: 1097–1098.

Csaba, G. and Kadar, M. (1978) Effects of epinephrine, glucagon and insulin on glucose metabolism of Planaria. *Endocrinologie*, **71**: 113–115.

Davis, K. T. and Shearn, A. (1977) Growth of imaginal disks from *Drosophila melanogaster*. *Science*, **196**: 438–440

El-Salhy M., Grimelius, L., Falkmer, S., Abou-Elella, R. and Wilander, E. (1980) Immunohistochemical evidence of gasteroenteropancreatic neurohormonal peptides of vertebrate type in the nervous system of a dipteran insect, the hoverfly. *Eristalis aeneus*. *Regulatory peptides* **1**: 187–204.

Gilbert, L. I and Kerkut, G. A. (1985) *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Pergamon press, Oxford, New York.

Ishaaya, I. and Swirski, E. (1970) Invertase and amylase activity in the armoured scales. *Crysomphalus aonidum* and *Aonidia aurantii*. *J. Insect. Physiol.* **16**: 1599–1606.

Ishay, J. (1975) Glucose levels in *Vespa Orientalis*, the effect of starvation *Comp. Biochem. Physiol.* **52A**: 91–96.

Ishay, J., Gilber, S., Galun, R., Doron, M and Laron, Zo (1976) The presence of insulin and some effects of exogenous insulin on hymenopteran tissue and body fluids. *Comp. Biochem. Physiol.* **54A**: 203–206.

Kramer, K. J., Tager, H. S and Child, C. N. (1986) Insulin like and glucagon like peptides in the insect haemolymph. *Insect. Biochem.* **10**. 179–192

Kramer, K. J., Chils, C. N., Speirs, R. D and Jacobs, R. M. (1987) Purification of insulin like peptides from insect haemolymph and Royal Jelly. *Insect. Biochem.* **12**: 91–98.

Lowry O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurements with Folin-phenol reagent. *J. Biol. Chem.* **93**: 265–275.

Lehninger, A. L. (1975) Biochemistry, Kalyani publishers, Original Printing Press, Ludhyiyana, India.

Mosna, G. (1981) Insulin can completely replace serum in *Drosophila melanogaster* cell culture *in vitro* *Experientia*, **37**: 466–467.

Pratt, G. E. Farnsworth, D. E. and Feyereisen, R. (1990) Changes in the sensitivity of adult cockroach corpora allata to brain allatostain, *Molec. Cell. Endocr.* **70**: 185–195.

Reitman, S and Frankel, S. (1957) Enzyme in blood. In: *Microanalysis, in Medical Biochemistry*. (I.D.P. Wooten ed) 4th Edition J. and A Churchill H. D. London 101–108.

Seifter, S., Dayton, S., Noic, D., Nuntwyler, E. (1950) The estimation of glycogen with anthrone reagent. *Arch. Biochem.* **25**: 191–200.

Stanchfield, J. E. and Yager, J. D. (Jr). (1979) Insulin effects on protein synthesis and secretion in primary cultures of amphibian hepatocytes. *J. Cell. Physiol* **100 (2)**.

Smith, E. L., Hill, R. L., Lehman, R., Leakqutz, R. J. Handler, R and White, A. (1983) Biochemistry of the endocrine system. Mammalian Biochemistry. In: Principles of Biochemistry, Mc Graw Hill. International Company, Singapore. 7th ed. 355–362.

Yu, C. G., Stay, B., Joshi, S and Tobe, S. S. (1993) Allatostamn content of Brain, corpora allata and haemolymph at different developmental stages of the cockroach, *diploptera punctata*: Quantification by ELISA and bioassay *J. Insect. Physiol.* **39**: 111–122.

Yui, R., Fijita, T and Ito, S. (1980) Insulin, Gastrin, Pancreatic polypeptide like immunoreactive neurons in the brain of silk-worm, *Bombyx mori*. Biomed Res. **1**: 42–46

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Effects of Theophylline and Methyl Anilino Derivatives on Common Stored Grain Pests

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Abstract: Insect sterilization property of 0.2% and 1% Theophylline in water and 0.2% newly synthesized Methyl anilino derivatives in chloroform were tested on two stored grain pests, the rice weevil (*Sitophilus oryzae*) and pulse beetle (*Callosobruchus chinensis*). Cell division in the testis, sperm population and F_1 population density were examined as parameters of effect. In *S. oryzae* 0.2% Theophylline decreased sperm count and population of F_1 generation without any effect on testicular cell multiplication; but in *C. chinensis* it increased appreciably sperm population having negligible effect on cell multiplication and F_1 population. Among the two methyl anilino compounds MAB reduced cell division and fertility in *S. oryzae* and cell division and sperm count in *C. chinensis*, whereas MAH reduced sperm count F_1 fertility in *S. oryzae* and only F_1 fertility in *C. chinensis*. The results reveal some population growth regulatory property of the compounds tested.

Keywords: Theophylline, Methyl anilino derivatives – MAB and MAH, *Sitophilus oryzae*, *Callosobruchus chinensis*, insect sterilization.

INTRODUCTION

Pest control by induced sterilization though is a very potential method but its application is limited because of lack of suitable sterilization procedures. Conventional chemosterilants available for easy sterilization are very unsafe and found to be carcinogenic. Search for suitable and acceptable chemical sterilants are going on. In this study phosphodiesterase inhibitor, theophylline and newly synthesized aniline compounds were tested on stored grain pests, rice weevil, *Sitophilus oryzae* and pulse beetle, *Callosobruchus chinensis* for their sterilization property.

MATERIALS AND METHODS

Rice weevil, *Sitophilus oryzae* and pulse beetle, *Callosobruchus chinensis* were used as experimental animals because of their severe pest status and easy rearing in the lab-

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oratory. Theophylline (dimethyl xanthine), a phosphodiesterase inhibitor was used in 1% and 0.2% water solution expecting physiological sterilization. Two newly synthesized methyl aniline compounds, (1) 1, 4, Bis–N–methyl anilino–2 butyne (MAB) and (2) 1, 6, Bis–N–methylanilino–2, 4 hexadyne (MAH) were supplied by Dr. K. P. Majumder of the Department of Chemistry, Kalyani University for testing their insecticidal property. The compounds being insoluble in water, 0.2% solution in chloroform was used. Higher concentrations were found to be lethal.

S. oryzae and *C. chinensis* were cultured in sterilized rice and black gram respectively. Newly emerged male and female insects were isolated and used for chemical treatment.

Topical treatment with chloroform solution were found to be lethal; hence both sexes of *S. oryzae* were released on partly eaten sterilized grains of limited number soaked with the chemicals and dried. Adult *C. chinensis* do not feed grains; so the treatment was restricted to pupa only which was unable to withstand topical treatment with alcohol solution. The treatment was done through film method. A dried film of 0.1 ml solution of the chemicals was made on a 3" petridish and 50 pupae picked up from infected grains were released in the chemical–coated petridish and kept till emergence. Newly emerged adults were sexed and used for study.

The parameters of induced sterilization examined were of three types (1) Gonadal cell-division, both mitotic and meiotic stages, (2) sperm population density and (3) fertility in F_1 generation.

For cell–division, the testis of the insects were taken out from adult males after 7 days of release in the chemically treated food. Slides were prepared following 0.7M KCl hypotonic pre–treatment, fixation by 1:3 aceto–alcohol and spreading in 60% acetic acid, air dried and stained with Giemsa solution. Both mitotic and meiotic figures were counted out of 600 available cells in each slide. Average of three such observations were compared with parallel control without chemical treatment.

Sperm count was done after 15 and 12 days of treatment respectively of *S. oryzae* and *C. chinensis*, as these were the days when maximum sperm–population was obtained. A pair of testes after dissecting out was homogenized in 0.5 ml Ringer solution and transferred on a haemocytometer. Average counts from 9 pairs of testes equally from 3 set of experiments were compared with parallel controls.

For population study in F_1 generation 10 adults of each sex of *S. oryzae* after 7 days of treatment were released in 20 gm of sterilized rice. Fifteen days after mating and oviposition the insects were removed. After 45 days, adults of F_1 generation emerged from infected grains which were counted and compared with control of F_1 population reared similarly but without chemical treatment. In case of *C. chinensis* the adults from treated pupae emerged within 6–7 days, sexed immediately and 10 of each sex were isolated in 200 sterilized grain seeds. The adults died after mating and oviposition, subsequently removed from the grains. Freshly emerged adults were counted to ascertain F_1 population.

RESULTS

Effects of theophylline

In *S. oryzae* theophylline solution of 0.2% had no effect on spermatogonial or spermatocyte cells leading to sperm development. Number of divisions obtained both in

treated and control insects had negligible difference. However, number of adult sperms and population in F_1 generation decreased respectively by 35.89% and 27.27%. Population reduction was not in confirmation with sperm reduction. After 1% theophylline treatment no adult survived to follow up the effects.

Increase in sperm production in *C. chinensis* by 0.2% theophylline solution was very significant, but it was not equally reflected in F_1 fertility which increased by only 5% against 61% increase in sperm population without any corresponding change of cell division index. Stimulatory effect of 1% theophylline on male germ cells of *C. chinensis* was more or less proportionately echoed in the increase of sperm population which rose by 12.8% against the 11.1% increase of cell division. F_1 fertility too correspondingly increased by 9.7% (Table 1).

Table 1. Effects of 0.2% and 1% theophylline on *S. oryzae* and *C. chinensis*

Name of sp.	Parameter of results	0.2% solution			1.0% solution		
		C.D.I.	S.C.	F_1 fertility	C.D.I.	S.C.	F_1 fertility
		30.30 (30.46)	20.9 (38.6)	40.0 (55.0)			
	% of reduction	0.525	35.889	27.272	Not viable		
S. oryzae	F. ratio treatment	0.01	58.05	3.358			
	C.D. at 5% level	3.410	4.823	16.058			
		22.0 (22.12)	37.3 (60.3)	268.0 (245.66)	24.74 (22.26)	60.7 (53.8)	269.3 (245.6)
	% of reduction	0.542	61.359	5.023	11.141	12.825	9.73
C. chinensis	F. ratio treatment	0.005.	13.90	2.077	1.725	2.290	41.650
	C.D. at 5% level	3.491	20.768	39.833	3.958	9.541	9.426

Figures in the parantheses indicated control, C.D.I. = Cell Division Index, S.C. Sperm count.

Effects of Methyl–anilino compounds

Methyl anilino compounds MAB and MAH in0.2% chloroform produced inhibitory effects in all the parameters studied. Germ cell division in the testes of both the insects was significantly inhibited by MAB compound, but the same effect was not significant by MAH. Reduction in germ cell division and sperm population was respectively 13.89% and 19.56% by MAB and in *c. chinensis* it was 15.27% and 23.29%. Reduction in germ cell division was more or less translated in sperm production. Corresponding decrease in F_1 population was not proportional to sperm decrease which was reduced

by only 9.42% in *S. oryzae* and 13.53% in *C. chinensis*.

Germ cell division is *S. oryzae* by MAH was reduced by 5.12%. On the contrary in *C. chinensis*, it was increased 7.04%. However, sperm population and corresponding F_1 population significantly decreased more or less proportionately in both the insects (Table 2).

Table 2. Effects of 0.2% solution of Methyl–anilino compounds in Chloroform on *S. oryzae* and *C. chinensis*

Name of sp.	Parameter of results	0.2% soln.of 1, 4–Bis–N (MAB) Methyl anilino–2 butyne			0.2% soln. of 6–Bis–N–methyl anilino–2, 4 hexadyne (MAH)		
		C.D.I.	S.C.	F_1 fertility	C.D.I.	S.C.	F_1 fertility
		23.02* (26.85)	44.4 (55.2)	47.66* (52.66)	25.34 (26.72)	54.6* (66.3)	32.66* (41.33)
	% of change	13.891	19.565	9.424	5.127	17.3	21.025
S. oryzae	F. ratio treatment	4.32	2.895	2.887	0.48	4.41	2.989
	C.D. at 5% level	3.58	13.282	3.926	4.17	11.58	5.351
		17.21* (20.31)	38.2* (49.8)	285.00 (330.00)	22.80 (21.30)	57.6 (66.6)	243.66* (290.33)
	% of change	15.271	23.293	13.536	7.042	13.513	16.081
C. chinensis	F. ratio treatment	11.22.	5.479	5.661	3.521	1.514	4.957
	C.D. at 5% level	1.95	10.471	48.26	1.618	15.305	33.098

Figures in the parantheses indicate control C.D.I. = Cell Divison Index, S.C. = Sperm Count.
*Significant at 5% level.

DISCUSSION

Theophylline is an inhibitor of the enzyme phospho–diesterase which controls intra–cellular C–AMP level. Cyclic adenosine monophosphate (C–AMP) is known to mimic insect diuretic hormone by stimulating water excretion (Casida *et al.* 1971). It also influences rate of metamorphosis in *Chironomous thummi thummi* by mimicing actions of morphogenetic hormones (Datta, 1975).

Theophylline is expected to increase intracellular C–AMP leading to increase or decrease of various metabolic activities. On this expectation theophylline was used to test its potential as a safe chemical sterilant. Muthukrishnan *et al.* (1979) observed larval mortality, in lepidopterain insect *Danaus chrysippus* after feeding 0.5% theophylline with food. Though Xanthine is also inhibitor of phosphodiesterase did not have any

deleterius effect on growth of the larvae of two noctuids, *Anticarsia gemmatalis* and *Spodoptera frugiperda*, but it produced synergistic effect with 0.5% allopurinol, an inhibitor of Xanthine oxidase (Slansky, 1993). Treatment of last stage nymph of *Dysdercus koenigii* with theophylline solution decreased fecundity and fertility. The effect lasted even in the post treated generations indicating disturbance in genetic material (Datta and Banerjee, 1978). In the present observation theophylline had no effect on the testicular cell division leading to spermatogenesis in any of the insects treated; but sperm population in *S. oryzae* decreased significantly indicating induction of partial sterility. However, in *C. chinensis* 0.2% theophylline increased sperm production and F_1 generation without any effect on spermatogonial and spermatocyte division. Stimulated post embryonic metamorphic changes have been reported in *Chironomus* sp. (Datta, 1975) by C–AMP. Increased sperm population without corresponding increase of germ cell multiplication may be due to stimulation of spermiogenic metamorphosis.

The two synthetic compounds MAB and MAH had inhibitory effects on all the parameters in both insects. *S. oryzae* was found to be more susceptible than *C. chinensis*. This was also the case with theophylline. *C. chinensis* was found to be more tolerant to aspirin and boric acid and sperm production was significantly inhibited even after negligible effect on testicular germ cell division (Datta and Bhattacharya, 1989). The pulse beetle seems to be more tolerant to the chemicals perhaps because of different food habits.

The compounds MAB, MAH have been derived from aniline compounds which are highly active and possess animal toxicity. If the compounds are dissociated in the insect body to produce amino methyl aniline it may result competitive inhibitor of catechol amines because of their structural similarity. There are various therapeutic uses of aniline compounds of which 9–aniline–acridine has anticarcinogenic effect (Baily *et al.* 1987). This anticarcinogenic effect of aniline compounds may occur for inhibition of spermatogonial cell division.

REFERENCES

Bailly, C., Bernier, J. L., Houssain, R., Helbeque, N. and Heinchart, J. P. (1987) Design of two metal chelating, DNA–binding models: molecular combination of pleomycin and amasacrine antitumour drugs. Anti Cancer, Drug, Design **1(4)**: 303–307.

Casida, J. E and Maddrell, S. H. P. (1971) Diuretic hormone release on poisoning *Rhodnius* with insecticide chemicals. Pesticide. Biochem. and Physiol. 1: 71–75.

Datta, S. (1975) Role of Cyclic–AMP on morphogenesis in *chironomus thummi thummi* (Kieffer). *Ind. J. Exp. Biol.*, **13(2)**: 191–192.

Datta, S. and Banerjee, P. K. (1978) Prostaglandins, Cyclic–AMP, U–7118 and acetic acid as insect growth regulators and sterilants. *Ind. J. Exp. Biol.* **15**: 880–883.

Datta, M. and Bhattacharya, A. (1989) Effects of Aspirin and Boric acid on the fertility control of *Sitophilus oryzae* and *Callosobruchus chinensis* Exp. Genetics, **5(1 & 2)**: 58–63.

Muthukrishnan, J., Madhaban, S. and Venkatasubba, K. (1979) Effect of Caffeine and theophylline and food utilization and emergence of *Danaus chrysippus* (Lepidoptera). Entomon **4(4)**): 307–312.

Slansky, F. Jr. (1993) Xanthine toxicity to caterpillers synergyzed by allopurinol, a xanthine dehydrogenase/oxidase inhibitor. *J. of Chemical Ecology*. **19(11)**: 2635–2650.

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Two new species of Agaonidae (Chalcidoidea: Hymenoptera) from *Ficus mollis* Vahl. (Moraceae)

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Abstract: Two new species of fig insects (family Agaonidae) viz. *Eupristina (Eupristina) mollis* (subfamily Agaoninae) and *Sycoscapter kathuriensis* (sf. Sycoryctinae) reared from the figs of *Ficus mollis* (family Moraceae) are described and compared with the other known species.

Keywords: Agaonidae, *Eupristina*, *Sycoscapter*, new species, *Ficus mollis*

INTRODUCTION

This paper provides descriptions of two new species of fig insects, viz, *Eupristina (Eupristina) mollis* belonging to the subfamily Agaoninae (Agaonidae) and *Sycoscapter kathuriensis* of the subfamily Sycoryctinae (Agaonidae), both reared from the figs of *Ficus mollis* (subgenus Urostigma, section Conosycea). Of these two wasps, *E. mollis* is the legitimate pollinator of the fig, while *S. kathuriensis* is an 'interloper' that breeds along with the pollinator.

Holotype and paratypes of the new species are kept in the Museum of Department of Zoology, University of Calicut (MZDC).

Eupristina (Eupristina) mollis sp.nov

Female: Length of head, thorax and apparent gaster 2.8 mm. Colour dark brown, eyes reddish, legs pale.

Head with a few setae on the face towards the eyes, slightly wider than long (8:7), almost twice the length of compound eye and 3 times the length of cheek; 3 ocelli, lateral ocelli positioned more close to eyes. Antenna (Fig. 1) 11 segmented, scape almost twice its width (15:8), pedicel 1/3 the length of scape and slightly longer than its width (10:9), bears a few axial spines; appendage of the 3rd segment acute, bears a few spines; 5th and 6th antennal segments bear five *sensillae linearia* each, the 7th bears 10; 8th, 9th and the 10th possess eight each, while the last segment carries six such sensillae. Maxillae as in Fig 2; labium reduced. Mandible (Fig. 3) slightly shorter

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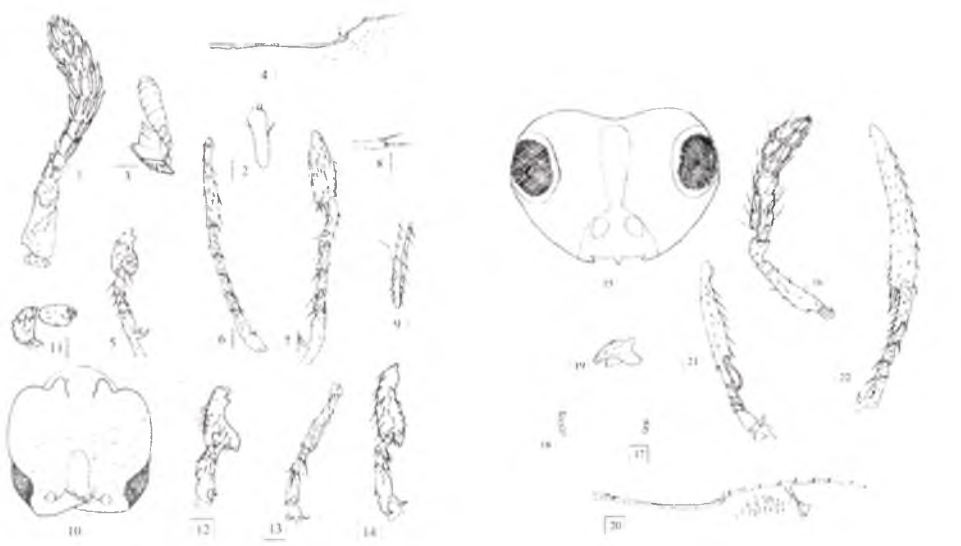

Figs. 1–14. *Eupristina mollis* sp. nov., Female: 1. Antenna, 2. Maxilla, 3. Mandible, 4. Fore wing, 5. Foreleg tibia and tarsus, 6. Midleg tibia and tarsus, 7. Hindleg tibia and tarsus, 8. Pygostyle, 9. Hypopygium Male: 10. Head, 11. Antenna, 12. Fore leg tibia and tarsus, 13. Midleg tibia and tarsus, 14. Hindleg tibia and tarsus.
Figs. 15–22. *Sycoscapter karthuriensis* sp. nov., Female: 15. Head, 16. Antenna, 17. Labial palpus, 18. Maxillary palpus, 19. Mandible, 20. Fore wing, 21. Foreleg tibia and tarsus, 22. Hindleg tibia and tarsus.

than wide (9:10), bidentate (one apical and the other subapical), only one gland visible, 3 ventral ridges; appendage twice as long as mandible, with 9 lamellae, the proximal 3 of which are used with the mandible and axially produced as teeth.

Thorax : Pronotum slightly setaceous, 1/4 its own width; measonotum with pollen pockets; pronotum, mesonotum and metanoto-propodeum with a length ratio 1:3:1; propodeal spiracles oval with a shallow notch on its inner side. Forewing (Fig. 4) 3 mm long, more than twice its width (7:3), slightly pubescent, venation obsolete beyond the marginal. Hindwing 2.8 mm long, venation not distinct. Foreleg, coxa with corbicula, almost twice its width; femur almost 3 times its width and 1.6 times the length of coxa; tibia (Fig. 5) 2/3 the length of coxa; its armature consists of a dorso–apical comb of 3 claws and a small ventral tooth; tarsus pentamerous, tarsomeres with the length ratio 2:1:1:3. Midleg coxa almost as long as wide and 1/3 the length of femur; tibia (Fig. 6) longer than femur; tarsus pentamerous, tarsomeres in the ratio 9:8:7:6:12. Hindleg, coxa with 12–14 robust setae; tibial armature (Fig. 7) consists of 2 bicuspid teeth on the ventral apex; tarsus pentamerous, tarsomeres with the ratio 6:4:3:2:4.

Gaster: Normal, protruding part of the ovipositor 1.7 mm long, slightly shorter than twice the length of gaster (4:7). Pygostyle (Fig. 8) with 2 long setae. Hypopygium as in Fig. 9.

Male: Length of head, thorax and apparent gaster 1.9 mm. Colour pale yellow.

Head (Fig. 10) slightly wider than long (10:9), 4 times the longitudinal diameter of the eyes; shortest distance between the antennal toruli and their distance to the inner margin of the compound eye in the ratio 3:1; facial groove reaching 1/3 the length of head. Antenna (Fig. 11) 4 segmented, 3rd segment annular, scape 1.5 times its own width; length of scape, pedicel and club in the ratio 4:2:5. Mandible bidentate, 2 glands.

Thorax, tergites fused. Foreleg, coxa almost as long as wide and 1/2 as long as femur; tibial armature (Fig. 12) consists of a dorsal comb of 4 blunt teeth and a ventral apical tooth; tarsus bimerous. Midleg, coxa 1/2 as long as femur and 1/3 the tibia; tarsus trimerous (Fig. 13), segments in the ratio 3:2:3. Hindleg, coxa as long as femur; tibial armature (Fig. 14) consists of a sharp dorsal tooth and a ventral comb of 4 teeth; tarsus trimerous, segments in the ratio 3:2:4 Gaster normal.

Host: *Ficus mollis* Vahl.

Holotype ♀, *India:* Karnataka, Bangalore, Coll. Prarthana Kathuria, May, 1994, ex. *Ficus mollis* Vahl., Paratypes 2 ♀♀and 1 ♂with same data as Holotype. Holotype and paratypes on slides nos. MZDC, A-XXVI/1, A-XXVII/1a and A-XXVII/2 respectively.

Note: This species is related to *Eupristina (Eupristina) belgaumensis* Joseph (1954). However they differ in the following characters. The female *E. (E). belgaumensis* has 40 axial spines to the antennal pedicel, 4 or 5 proximal lamellae of mandibular appendage are produced to teeth and midtarsus is shorter than tibia. But in *E. (E.) mollis* there are only 16–20 axial spines to the antennal pedicel, only 3 lamellae of mandibular appendage are produced to teeth and midtarsus is distinctly longer. In male *E. (E.) belgaumensis* mid and hind tarsi are pentamerous, while in *E. (E) mollis* they are trimerous.

***Sycoscapter kathuriensis* sp. nov**

Female: Length of head, thorax and apparent gaster 3.4 mm. Gastral tail 5.6 mm. Colour iridescent green, legs pale and antenna smoky brown.

Head (Fig. 15) distinctly wider than long (4:3). Compound eyes 3/5 the length of head and slightly longer than the cheek; OOL: POL 1:4; antennal toruli situated at the lower third of the face, at a distance twice its diameter from the epistomal margin; the shortest distance between the toruli 1/4 their minimum distance to the inner margin of the eyes. Antenna (Fig. 16) 11 segmented, scape a little over 4 times its own width and almost three times the pedicel; 1st funicular segment and club segments bear 4 and other funicular segments bear 3 each *sensillae linearia*; all funicular segments bear a basal row of long setae. Labial palpus (Fig. 17) 2 segmented (3:5) and maxillary palpus (Fig. 18) 4 segmented in the ratio 2:4:2:5. Mandible (Fig. 19) bidentate.

Thorax: pronotum 1/5 the length of mesonotum; notauli distinct only in the anterior half of scutum; metanotum dorsally visible. Forwing (Fig. 20) 2.5 mm. long; stigmal knob 1/3 the length of stigmal vein, 16–18 robust hairs below the marginal vein and 4 on the stigmal vein; submarginal, marginal, stigmal and postmarginal veins in the ratio 8:5:6:3. Hindwing 1.2 mm long. Foreleg coxa twice its width and slightly longer than the combined length of femur and trochanter; tibia (Fig. 21) 1.3 times as long as tarsus; tibial armature consists of two small apical teeth (one dorsal and the other ventral) and

the long curved ventral spur, the tip of which reaches the middle of the 2nd tarsomere; tarsus pentamerous, tarsomeres with the ratio 10:5:4:2:11. Midleg tibia twice as long as the combined length of femur and trochanter and 1.5 times as long as tarsus; tarsal ratio 15:7:7:6:11. Hindleg, coxa almost twice its width and a little shorter than the combined length of femur and trochanter, which is a little shorter than the length of tibia (Fig. 22); tarsal ratio 3:8:7:6:10.

Abdomen: ovipositor valves 3.5 times the apparent gaster.

Male: not known.

Host: *Fiscus mollis* Vahl.

Holotype ♀ *India:* Karnataka, Bangalore, Coll. Prarthana Kathuria, May, 1994, ex. *Ficus mollis* Vahl., Paratype ♀, same data as Holotype, mounted on slides nos. MZDC, E-XXVII/3 and E-XXVII/3a respectively.

Note: This species is related to *Sycoscapter triformis* Joseph (1957). But *S. kathuriensis* is easily distinguishable by the reduction of one funicular segment to the antenna and the presence of about 18 robust hairs below the marginal vein instead of 9 in *S. triformis*.

This species is named after Ms. Prarthana Kathuria (Gandhi Krishi Vignan Kendra, University of Agricultural Sciences, Bangalore) in recognition of her keen interest in *Ficus*–wasp interaction.

ACKNOWLEDGEMENTS

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REFERENCES

Joseph, K. J. (1953) Contributions to our knowledge of fig insects (Chalcidoidea: Parasitic Hymenoptera) from India. VI-On six species of Agaoninae., *Agra. Univ. J. Res. Sci.*, **2**: 267–284.

Joseph, K. J. (1957) *Idem.* VII. Description of fifteen new and revision of some old species. *Ann. Soc. Ent. France*, **125**: 7–133.

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Development of female accessory sex glands on implanted male genital disc and its suppression by testis in *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae)

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Abstract: The genital disc of male third (last) instar larvae of *Oryctes rhinoceros* was implanted into 0-day old female pupa. On adult emergence, the implanted genital disc got transformed to female accessory gland (II pair), instead that of males. However, when testis of third instar larvae in which cellular epithelium and spermatogonia are differentiated were implanted along with genital disc, such a transformation was found to be suppressed. Neither the genital disc nor the testis of early stages of this insect were competent enough to bring forth the respective changes. The present study reveals that male genital disc is not strictly programmed for the development of male characters alone but it is likely to be transformed to female organs if they are kept in female body. This study also highlights the androgenic function of testis which is expedited only after its cellular differentiation.

Keywords: Male genital disc, female accessory gland, implantation, suppression, testis, *Oryctes rhinoceros*

INTRODUCTION

Accessory reproductive glands in insects perform vital functions during reproduction and development. Their development from genital imaginal disc provide outstanding models for the analysis of cell biology, endocrinology and genetics (Kaulenas 1992). According to Madhavan and Schneiderman (1977), the genital disc rudiment first becomes detectable as a group of about 60 cells in the late embryos of *Drosophila*, and in the newly hatched larva the rudiment is seen as a clump of cells in front of the anal opening. Clonal analysis indicates that about 11 cells become committed as male genital disc at blastoderm stage (Schubach *et al* 1978). Analysis of gynandromorph in *Drosophila* strongly suggest that for the development of male genital disc, the male primordia and the primordia of analia develop together in the larva as a single disc

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while female primordium is suppressed (Epper and Nothiger 1982). The male accessory glands in *Oryctes rhinoceros* consist of a pair of tubular structures arising at pupal stage as outgrowth of ectoderm cells of ampullae, the anterolateral part of the genital disc (Jacob 1989). However, in the females there are two pairs of accessory glands which are also differentiated at pupal stage (Geetha 1991). One pair is ovoid and yellowish while the other is ovoid and brown coloured and they are attached to the genital pouch. In the present study, an attempt is made to investigate whether the male genital disc of *Oryctes rhinoceros* is programmed for the development of male structures alone or it is liable to changes and to trace whether the testis before and after its cellular differentiation has any androgenic function in maintaining maleness.

MATERIALS AND METHODS

Larvae of *Oryctes rhinoceros* were collected from local manure pits and were reared in the laboratory on sterilized cow dung as reported earlier (Jacob 1989). O-day female pupae and male larvae of various stages were separated from the stock colony and were surface sterilized in 70% alcohol. 2 and 16 day old I and II instar and 2 and 35 days old III instar male larvae were used for the experiments. An incision was made on the midventral part of the 9th abdominal sternite of the larvae and the genital disc alongwith the surrounding tissues were removed and kept in insect saline. A longitudinal slit was made on the tergite of 7th and 8th abdominal segment of the female pupa after chilling for a minute. The genital disc was inserted through the slit into female pupa. The wound was sealed with wax after applying antibiotics. The pupae were kept in sterilized bottles and were watched constantly for any contamination. On adult emergence, *i.e.*, after 16–18 days, the implanted organs were recovered by opening the sealed portion. Male genital disc of I, II and III instar larvae were implanted as described above. In the second sets of experiments, testis of various stages were also implanted alongwith genital disc. Testis rudiments are dorsally placed and seen embedded in the fat body surrounding 7th and 8th segments and are connected to genital disc with long ducts. For histological studies, the implants and female (normal) accessory glands were fixed in Bouin's fluid and stained with Heidenhain's haematoxylin eosin.

RESULTS AND DISCUSSION

Results of the experiments are given in Table 1. Genital disc of early and late stages of the male larvae were implanted into the body of female pupa. Male genital disc of *Oryctes rhinoceros* is a triangular organ attached to 9th abdominal segment, with two lateral arms, the ampullae into which *vas deferens* and a nerve from a last abdominal ganglion enter (Fig. 1). The middle portion of the genital disc lodges a chitinous plate having a triangular shape which helps in the identification of larvae. Accessory glands develop from the ectodermal cells proliferating from the ampullae dorsal to *vas deferens* during pupal stage. The chitinous plate is ejected alongwith the last larval exuvium and thus it is not seen in pupa (Jacob 1989). The implanted genital disc as well as the testis were all covered by tracheae of the female pupa (host). The implants from I and II instar larvae had no remarkable changes. It appeared that the implants either genital disc or testis, were of very early stages and they were not competant

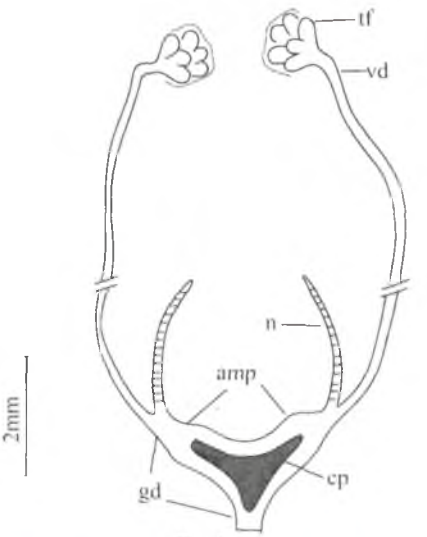

Fig. 1.

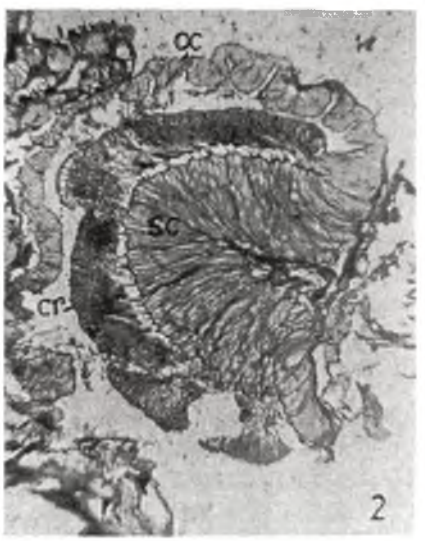

Fig. 2.

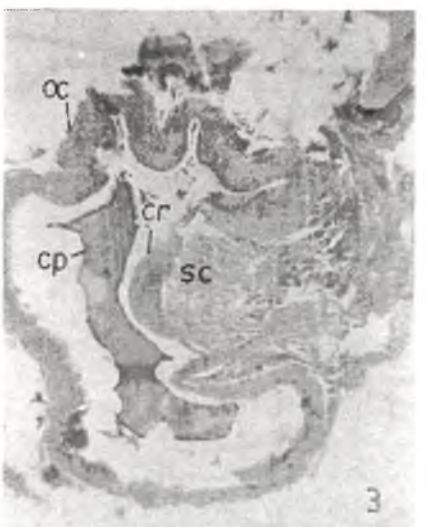

Fig. 3.

Fig. 1 Camera lucida sketch of the genital disc and associated ducts, and testis rudiments in III instar larva.
Fig. 2. Sagittal section through the histological preparation of normal II pair of female accessory gland in *O. rhinoceros* × 150.
Fig. 3. Sagittal section through the histological preparations of implanted genital disc after recovery from female × 150. **Abbreviations used:** amp - ampullae, cp - chitinous plate, cr - chitinous rods, gd - genital disc, oc - outer covering, sc - secretory cells. n - nerve, tf - testis follicles; vd - vas deferens.

enough to undergo differentiation inspite of the fact that female body is an excellent endocrine pool. Gallois (1989) reported that in *Locusta migratoria*, the rudiments of accessory glands from early larvae did not undergo any changes when implanted into adult. It was probably because they had to reach a critical period for differentiation.

Table 1. Showing details of implantation of male genital disc to 0 day old female pupa and appearance of the implant on recovery from female adult

Sl. No.	Stage of the insect Instar	Day	Type of implant Genital disc (GD)	Testis (T)	Nature of the implant on recovery from from female adult
1.	I	2	GD		The implants were entangled in tracheae and fat body of the host. No development occurred
			GD	T	
		16	GD		
			GD	T	
2.	II	2	GD		do
			GD	T	
		16	GD		do
			GD	T	
3.	III	2	GD	–	The GD was entangled in tracheae and fat body of female adult, a bean shaped brown coloured organ like II pair Accessory glands of female insect.
			GD	T(2)	do
			GD	T(35)	GD and testis are entangled in tracheae and fat body, brown chitinous plate was missing, GD and testis were enlarged in size.
		35	GD		GD was entangled in tracheae and fat body, bean shaped, brown coloured organ like II pair of accessory glands in females.
			GD	T(2)	do
			GD	T(35)	GD and testis were glands in tracheae and fat body, chitinous plate was missing in GD, GD and testis was enlarged in size

T(2) and T(35), testis of 2nd and 35 day old third instar larvae

In *Oryctes rhinoceros*, the genital disc from early and late III instar larvae implanted into female 0-day pupa were enlarged in size and got folded into a bean shaped brown organ totally covered in the tracheae of newly emerged female adult. It seemed that the chitinous plate had covered the whole organ. However, in the normal animals, the chitinous plate is ejected along with the larval exuvium at pupal moult (Jacob 1989). The implants on detailed study showed morphological appearance of female accessory gland (II pair). Histological studies showed that it is double layered cup–like organ. The outer wall was irregular in shape with long columnar cells. The inner layer was made of differentiating chitinous rods appeared to develop from chitinous plate of male genital disc. The centre was filled by transforming secretory cells (Fig. 2). The morphological and histological similarities suggest that the implanted male genital disc was getting transformed to second pair of female accessory gland. Geetha (1991) reported two pairs of female accessory glands attached to the genital pouch of female *Oryctes rhinoceros* which get differentiated in the pupal stage. Female accessory gland (II pair) is ovoid and brown in colour with an outer irregular

wall made of well defined columnar cells and an inner row of chitinous rods, with prominent secretory cells in the centre (Fig. 3). In the present study, though not well defined, the implanted genital disc also elicited a similar structure. It may be because the male genital disc is not strictly programmed for the development of male organ but is liable to hormonal changes. Thus when they were implanted into female body naturally they developed to female organ. Moreover, this transformation was noticed only in the genital disc of third instar larva probably because it was the critical period of differentiation and it seems to be hormonally controlled. Many experiments carried out in larvae and embryos of *Drosophila*, their gynandromorph analysis, on the development and fate of genital disc suggest that the genital disc in its origin is common to both sexes and if male primordium is to be expressed the female is suppressed and *vice versa* (Epper and Nothiger 1982, Littlefield and Bryant 1979, Kaulenas 1992).

It is interesting to notice that a differentiated testis of third instar (late) larva of *Oryctes rhinoceros*, could suppress the development of female accessory gland on implanted male genital disc. This strongly suggest that there is some endocrine principle in the testis which helps to maintain maleness in normal insect. In the I, II and early III instar larvae, primordial germ cells filled the six testicular follicles. But in the late third instar larvae, primary spermatogonium and a cellular epithelium get differentiated in the testis (Jacob 1989). The *in vitro* studies of spermatogeneis in*Oryctes rhinoceros* revealed that the testis sheath has a major role in maintaining the process of spermatogenesis as the spermatogonia without testis sheath degenerated in the medium (Jacob 1992). The electrophoretic studies of the culture medium before and after testis incubation gave more evidence that testis sheath is secretory (Jacob 1996). In *Heliothis virescens* also spermatogenesis was promoted in presence of testis sheath (Loeb *et al* 1988). Loeb (1991) further reported that isolated sperm duct in the culture medium developed to a normal tube in the presence of testis sheath and fat body. Gundevia and Ramamurthy (1974) suggested that the apical cells or Verson's cells associated with spermatogonia have some endocrine function. However, Menon (1969) adduced evidence through electronmicroscopic studies on apical cells in testis of *Tenebrio molitor* that it had no endocrine function. Recent studies on the testis sheath of *Oryctes rhinoceros* (electron microscopic studies, unpublished data) show secretory granules in septa and sheath covering spermatogonia which is not seen in early larval and late adult testis. It is thus assumed that the testis sheath has some androgenic (endocrine) function to express maleness. The present study also shows that genital disc from male larvae is not strictly programmed for male development. Moreover, there is a critical time in the larval development for differentiation gonads, which in turn is hormonally controlled.

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REFERENCES

Epper, F. and Nothiger, R. (1982) Genetic and developmental evidence for a repressed genital primordium in *Drosophila melanogaster*; *Dev. Biol* **94**: 163–175.

Gallois, D. (1989) Control cell differentiation in the male accessory reproductive glands of *Locusta migratoria* aquisation and reversal of competence to imaginal secretion. *J. Insect. Physiol.* **35**: 189–195.

Geetha, P. R. (1991) Studies of some aspects of female accessory reproductive glands in *Opisina arenosella (Nephantis serinopa)* Cryptophasidae: Lepidoptera and *Oryctes rhinoceros* (Scarabaeidae: Coleoptera) Ph.D. thesis submitted to the University of Kerala, Trivandrum.

Gundevia, H. S. and Ramamurthy, P. S. (1974) Verson's cells and aberrant spermatozoa of *Hydrophilus olivaceus* Fabr. (Coleoptera: Hydrophilidae) *Int. J. Insect Morphol. Embryol.* **3**: 121–125.

Jacob, M. (1989) Differentiation of male reproductive system in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) *Proc. Indian Acad. Sci.* **98**: 233–242.

Jacob, M. (1992) *In vitro* spermatogenesis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). The role of ecdysone and juvenile hormone. *Zool. Sci.* **9**: 457–461.

Jacob, M. (1996) Role of testis sheath and 20–hydroxy ecdysone during *in vitro* spermatocytogenesis in the coleopteran beetle *Oryctes rhinoceros*. *Indian. J. Exp. Biol.* **34**: 572–574.

Kaulenas, M. S. (1992) Insect Accessory reproductive structures. Function, structure and development. Zoophysiology Vol. 31 (Eds. S. D. Bradshaw *et al*) Springer Verlag, Berlin, New York, London, 224.

Littlefield, C. L. and Bryant, P. J. (1979) Prospective fates and regulative capacities of fragments of the female genital disc of *Drosophila melanogaster*. *Dev. Biol.* **70**: 127–148.

Loeb, M. J. (1991) Development of isolated spermducts from *Heliothis virescens*. *Inver. Repro. Develop.* **20**: 67–73.

Loeb, M. J., Brandt, E. P., Woods, C. W., and Bell, R. A. (1988) Secretion of ecdysteroid by sheath of testis of the gypsy moth and its regulation by testis ecdysiotropin. *J. Exp. Zool.* **248**: 94–100.

Madhavan, M. M. and Schneiderman, H. A. (1977) Histological analysis of the dynamics of growth of imaginal disc and histoblast nest during the larval development of *Drosophila melanogaster*. Wilhelm Roux's *Arch. Dev. Biol.* **183**: 269–305.

Menon, M. (1969) Structure of apical cells of testis of tenebroid beetle *Tenebrio molitor* and *Zophabas rugipes*. *J. Morphol.* **127**: 409–429.

Schupbach, T., Wieschaus, E. and Nothiger, R. (1978) The embryonic organization of the genital disc studies in genetic mosaics of *Drosophila melanogaster*. *Wilhelm Roux's Arch. Dev. Biol.* **185**: 249–270.

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Feat of Pure breeds and Single cross hybrids of *Bombyx mori* L.

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Abstract: Among the different silkworm breeds and hybrids the pure race NB_4D_2 performed better in respect of larval duration (24.1 days), single cocoon weight (1.7 g), pupal weight (1.4 g), shell weight (0.327 g), filament weight (0.299 g) and hatching per cent (93.0%) while the Pure Mysore (PM) had lower values for most of the economic characters. Of the selected multi × bivoltine hybrids, $MW_2 \times NB_4D_2$ performed better in all the economic characters except for denier and hatching percentage. When MW_2 was used as paternal parent the performance of the cross was affected.

Keywords: Silkworm breeds, pure race NB_4D_2 bivoltine hybrids

INTRODUCTION

South India, enjoys favourable climatic conditions throughout the year for mulberry cultivation as well as silkworm rearing. At present, a large number of hybrid combinations with Pure Mysore (PM) as female parent and improved bivoltine races as male components are being reared. Since Mysore white-2 (MW_2) has shorter larval period, higher silk content, high disease resistance, better silk quality and higher silk yield this can be used in place of PM in the preparation of hybrid combination for commercial use. The present study is an attempt to examine the performance of some selected single cross hybrids of silkworm *Bombyx mori* L. involving MW_2 as both maternal as well as paternal breeds as information is scanty in this regard.

MATERIAL AND METHODS

The silkworms were cultured as per Krishnaswami (1978). The four genetically divergent pure races viz., PM, MW_2, NB_4D_2 and NB_{18} were utilised for this experiment.

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Direct single crosses and reciprocals were effected between multi and bivoltines and eggs were prepared by adopting standard grainage techniques. Six hundred worms of each race/hybrid were taken immediately after second moult after giving first feed and divided into three replications of 200 worms each. Circular bamboo trays (75 cm dia.) were used and treatments were allocated randomly so as to follow complete randomised design. The standard method for young and late age silkworm rearing was practiced by feeding leaves of M-5 variety of mulberry. Observations were made for egg, larval, pupal, cocoon and silkcharacters. The data collected was statistically analysed using completely randomised block design as described by Panse and Sukhatme (1967). Duncan's multiple range test (DMRT) was applied for comparing treatment means. Letter designation was applied for comparing the treatments. In the table means between treatments followed by a common letter are not statistically different at 5 per cent level by DMRT. For brevity data reduced to single decimal.

RESULTS

The mean performance of selected single cross hybrids using pure races (PM, MW_2, NB_4D_2 and NB_{18}) both as maternal and paternal parents was evaluated (Table 1).

Among the pure races studied the PM parental breed showed maximum larval duration of 29.2 days while the MW_2 had minimum of 22.4 days. Out of their hybrids it was very interesting to observe that $MW_2 \times NB_4D_2$ had the minimum larval duration of 23.3 days whereas $NB_4D_2 \times$ PM cross had maximum larval duration of 26.7 days which was on par with that of $MW_2 \times NB_{18}$ and MY_1. Of the pure races, NB_{18} showed maximum larval weight of 39.7 g (weight of ten larvae) while the PM breed had only 18.3 g. Of the crosses, $MW_2 \times NB_4D_2$ weighed 42.6 g and was superior to all the pure races and their hybrids. However, $NB_4D_2 \times MW_2$ registered lowest of 24.4 g which was on par with Mysore Yellow-1(MY_1). The pure race MY_1 showed highest survival of 90.5 per cent which did not differ from ruling hybrid, PM $\times$ NB_{18} (89.8) and $MW_2 \times NB_4D_2$ (87.5), PM $\times$ NB_4D_2 (85.8) and PM (85.2) in respect of survival. However, lowest survival of 74.0 per cent was effected by $NB_4D_2 \times MW_2$.

The MY_1 race gave maximum cocoon number (181.0) and was on par with NB_4D_2 (161.3), NB_{18} (165.0), PM $\times$ NB_{18} (179.7), $MW_2 \times NB_{18}$ (170.7), PM $\times$ NB_4D_2 (171.7) and $MW_2 \times NB_4D_2$ (161.7). However, $NB_{18} \times$ PM cross had the minimum (111.7) of cocoons.

Among the different races and hybrids studied 200 cocoons of PM $\times$ NB_{18} weighed 292.4 g and was on par with PM $\times$ NB_4D_2 (291.5) and $MW_2 \times NB_4D_2$ (284.2 g). However, $NB_{18} \times$ PM recorded the lowest yield of 179.2 g. Among the different pure races minimum (113.9) and maximum (242.3 g) cocoon weight was recorded in PM and NB_{18} respectively. Single cocoon weight of 1.9 g was recorded by $MW_2 \times NB_4D_2$ which differed significantly from all other pure races and hybrids. The hybrid, $NB_4D_2 \times$ PM showed the lowest of 1.4 g. Among the pure races NB_4D_2 ranked first by weighing 1.7 whereas PM ranked last with only 1.1 g. Similar to the cocoon weight, $MW_2 \times NB_4D_2$ cross had maximum pupal weight of 1.5 g and showed variation with all other pure races and hybrids. $NB_{18} \times$ PM produced the lowest of 1.2 g. NB_4D_2 had 1.4 g while the PM weighed only 0.9 g. As in case of pupal weight $MW_2 \times NB_4D_2$ resulted with highest shell weight of 0.362 g and it differed from all other pure races and hybrids. $NB_4D_2 \times$ PM weighed lowest with 0.229 g. Among the pure races, NB_4D_2 effected maximum shell weight of 0.327 while lowest of 0.126 g was seen in PM

Table 1. Performance of few pure races and hybrids of *B. mori* L.

Breeds/ hybrids	Larval duration (days)	Weight (g/ten larvae)	ERR (%)	Cocoon yield/200 larvae (No.) (g)	Weight (g)	Single cocoon wt.(g)	Single pupal wt.(g)	Single shell wt.(g)	Shell percent (%)	Filament length (m)	Filament wt.(g)	Denier	Fecundity (egg/ laying	Hatching percent (%)
PM	29.2^{a}	18.3^{f}	85.2^{abcd}	113.7^{de}	113.9^{e}	1.1^{h}	0.9^{g}	0.126^{f}	11.5^{e}	422.6^{h}	0.107^{g}	2.3^{e}	369^{f}	90.6^{cde}
MY_1	23.6^{fg}	23.2^{f}	90.5^{a}	181.00^{a}	237.5^{c}	1.4^{fg}	1.2^{ef}	0.170^{b}	12.2^{e}	497.7^{g}	0.139^{f}	2.5^{de}	545^{c}	91.1^{bcde}
MW_2	22.4^{h}	28.8^{b}	82.2^{cde}	136.3^{cd}	165.5^{d}	1.3^{g}	1.1^{f}	0.189^{g}	14.7^{d}	591.4^{f}	0.150^{f}	2.3^{e}	536^{d}	89.6^{e}
NB_4D_2	24.1^{ef}	36.7^{a}	80.8^{cde}	161.3^{ab}	165.9^{d}	1.7^{d}	1.4^{bc}	0.327^{b}	18.6^{ab}	1027.6^{a}	0.298^{ab}	2.6^{cd}	608^{ab}	93.0^{abcde}
NB_{18}	25.1^{d}	39.7^{b}	82.5^{bcde}	165.0^{ab}	242.3^{c}	1.5^{de}	1.2^{ef}	0.291^{c}	18.9^{a}	1043.9^{a}	0.259^{c}	2.2^{e}	620^{a}	92.7^{abcde}
$NB_{18} \times$ PM	24.5^{e}	39.0^{b}	75.8^{g}	117.7^{e}	179.2^{d}	1.6^{cde}	1.2^{ef}	0.293^{c}	16.0^{c}	722.3^{de}	0.208^{e}	2.6^{cd}	466^{e}	93.1^{abcde}
PM $\times$ NB_{18}	25.3^{d}	31.3^{g}	89.8^{ab}	179.7^{ab}	292.4^{a}	1.5^{ef}	1.2^{ef}	0.246^{e}	16.4^{c}	828.8^{c}	0.264^{c}	2.9^{ab}	603^{ab}	94.3^{ab}
$MW_2 \times NB_{18}$	23.4^{g}	34.9^{de}	85.3^{abcd}	170.7^{ab}	244.6^{c}	1.6^{cd}	1.3^{bcd}	0.295^{c}	18.1^{ab}	848.4^{b}	0.261^{c}	2.8^{abc}	576^{bc}	95.9^{a}
$NB_{18} \times MW_2$	25.1^{d}	39.9^{b}	81.5^{cde}	156.3^{bc}	250.3^{c}	1.7^{bc}	1.4^{bc}	0.320^{b}	18.5^{ab}	891.9^{b}	0.288^{b}	2.9^{ab}	604^{ab}	90.2^{de}
$NB_4D_2 \times$ PM	26.7^{b}	36.4^{cr}	78.3^{de}	130.0^{de}	229.1^{c}	1.4^{f}	1.3^{cd}	0.229^{f}	16.1^{c}	682.1^{e}	0.207^{e}	2.7^{bc}	550^{cd}	90.1^{de}
PM $\times$ NB_4D_2	26.2^{c}	34.5^{ef}	85.8^{abcd}	171.7^{ab}	291.5^{a}	1.7^{bc}	1.4^{b}	0.268^{d}	16.1^{c}	777.9^{cd}	0.235^{d}	2.7^{bcd}	596^{ab}	94.0^{abc}
$MW_2 \times NB_4D_2$	23.3^{g}	42.6^{a}	87.5^{abc}	161.7^{ab}	284.2^{ab}	1.9^{a}	1.5^{a}	0.362^{a}	18.6^{ab}	1080.8^{a}	0.318^{a}	2.6^{cd}	595^{ab}	90.1^{de}
$NB_4D_2 \times MW_2$	25.2^{d}	24.4^{f}	74.0^{f}	134.7^{cde}	254.6^{bc}	1.7^{bcd}	1.4^{b}	0.290^{c}	17.6^{b}	863.1^{b}	0.288^{b}	3.0^{a}	549^{d}	83.1^{f}
CD (P=0.05)	0.5	1.5	7.6	23.7	33.5	0.12	0.12	0.01	1.0	62.3	0.02	0.2	33.9	3.5

Means followed by same letter in a column are not significantly different by least difference criterion (P=0.05). For brevity data reduced to single decimal

breed. The NB_{18} race showed the highest shell per cent (18.9) but was on par with NB_4D_2 (18.6), $MW_2 \times NB_{18}$ (18.1), $NB_{18} \times M_2$ (18.5) and $MW_2 \times NB_4D_2$ (18.6). Lowest of 11.5 and 16.0 per cent was effected by PM and $NB_{18} \times PM$ respectively in case of pure race and crosses.

It was very interesting to observe that $MW_2 \times NB_4D_2$ effected the highest filament length of 1080.8 and was on par with NB_{18} (1043.9) and NB_4D_2 (1027.6 m). PM used as pure (422.6) or as paternal parent with NB_4D_2 produced lowest filament length (682.1 m). Similar to filament length, $MW_2 \times NB_4D_2$ resulted in filament weight of 0.318 and was on par with NB_4D_2 but differed significantly from all pure races and hybrids. $NB_4D_2 \times PM$ and PM had lowest filament weights of 0.207 and 0.107 g respectively. The bivoltine race NB_{18} was superior with denier as low as 2.2 which was on par with MW_2 and PM breed. However, among the crosses $NB_{18} \times PM$ (2.6) and $MW_2 \times NB_4D_2$ (2.6) showed no variation.

The pure race NB_{18} produced as many as 620 eggs which was on par with that of NB_4D_2 (608), $NB_{18} \times MW_2$ (604). However, the PM among multivoltines and $NB_{18} \times PM$ among the crosses laid lowest of 369 and 446 eggs/female respectively. Cross breed, $MW_2 \times NB_{18}$ effected maximum hatching per cent of 95.9 which was followed by $PM \times NB_{18}$ (94.3), $PM \times NB_4D_2$ (94.0), $NB_{18} \times PM$ (93.1), NB_4D_2 (93.0) and NB_{18} (92.7). However, $NB_4D_2 \times MW_2$ showed the lowest hatching per cent of 83.1.

Among the pure races NB_4D_2 performed better in respect of larval duration (24.1 days), single cocoon weight (1.7 g), pupal weight (1.4 g), shell weight (0.327 g), filament weight (0.298 g) and hatching per cent (93.0) while the PM had lower values for most of the economic characters. The hybrid, $MW_2 \times NB_4D_2$ performed better in all the economic characters except for denier and hatching percentage than other tested hybrids. The performance of the cross was affected wherever MW_2 was used as paternal parent.

DISCUSSION

In general the hybrids performed better than the pure races in respect of the characters studied. NB_4D_2 among the pure races and $MW_2 \times NB_4D_2$ among the single crosses tested performed better. Earlier reports are also in line with the present finding. Yokoyama (1957, 1974, 1979) and Kovalev (1970) reported that pure races were inferior to their F_1 hybrids. Krishnaswami and Tikoo (1972) recorded better performance of C-Nichi, HS-6, J-112, C-108 and KA than the PM race as pure crop and recommended for the use of these exotic races both as male and female parents in the production of hybrid seed. Bivoltines, NB_4D_2, NB_3C_1 and NB_1C_1 performed better than KA, NN6D (Anonymous, 1975). Studies conducted by Benchamin and Krishnaswami (1980) with multivoltine and four bivoltine races indicated that NB_4D_2, NB_7, NB_{18} and KA had high egg production index. The pure lines CB_2 and CB_5 were inferior to their hybrids (Datta *et al.*, 1981) recording survival per cent of 52.6 as against 75 to 80 per cent in hybrid combination. However, Mathur *et al.* (1988) reported that out of six pure races studied KA proved most hardy (80.7% survival) but silk ratio was as low as 16.1 as compared to 21.0 per cent in case of NB_7. Thangavelu (1988) who tested the performance of PM, NB_4D_2, NB_{18} and cross breeds concluded that pure races performed inferior to cross breeds under tropical conditions.

Datta (1984) reported better performance of NB_7 and NB_{18} in combination with new multivoltine races viz., MY_1, MY_2, MW_1 and MW_2 in almost all commercial characters compared to PM × NB_{18} and PM × NB_7 whereas they performed better with CA_2 as compared to old races like NB_7 and NB_{18} (Anonymous, 1985a) and MW_1 × CA_2 gave the highest yield per 10,000 worms (17.7 kgs) followed by $MW_1 \times NB_4D_2$ (16.7 kgs) (Anonymous, 1985b). In a multilocational trail MY_1 × bivoltine hybrid performed better with respect to yield, return per 100 dfl's as compared to existing PM × bivoltine hybrids (Anonymous, 1986). Nagaraju *et al.* (1987) reported that MY_1 × bivoltine hybrids performed better than "Pure Mysore hybrids" in large scale trail with farmers of Karnataka.

REFERENCES

Anonymous, (1975) Silkworm Breeding and Genetics section, *Ann. Report.* CSR and TI. Mysore, 37–38.

Anonymous, (1985a) Evolution of new multivolting breeds, MY_1 and MY_2 *Ann. Report.* CSR and TI. Mysore, 8–10.

Anonymous, (1985b) Multilocational trails of the newly evolved breeds and their hybrids. *Ann. Report.*, (CSRFTI), Mysore, 15.

Anonymous, (1986) Identification of hardy bivoltine combinations. *Ann. Report*, CSR and TI, Mysore. 3–4.

Benchamin, K. V. and Krishnaswami, S. (1980). Studies on the Egg production Efficiency in silkworm, *Bombyx mori* L. (i) on the various factors contributing to egg production. *Proc. Seric. Symp. Sem.* Coimbatore. 1–6.

Datta, R. K., Das, S. K., Pal, D. E. and Sengupta, K. (1981) Evolution of superior multivoltine breeds through breeding technique. *Proc. Seric. Symp. Semi* (1980), TNAU, Coimbatore 39–42.

Datta, R. K. (1984) Improvement of Silkworm races. (*Bombyx mori* L.) in India. *Sericologia* **24(3)**: 393–415.

Kovalev, P. A. (1970) Silkworm Breeding Stocks. Central Silk Board, Bombay, 223.

Krishnaswami, S., and Tikoo, B. L. (1972) A comparative study of the performances of pure races currently under rearing in Mysore State. *Ind. J. Seric.* **10**: 66–69.

Krishnaswami, S. (1978) New technology of silkworm rearing II. *Indian Silk* **16**: 7–15.

Mathur, Jyothi, P., Tyagi, R. and Verma, M. B. (1988). Performance of some pure and hybrid races of *Bombyx mori* in South Rajasthan (Udaipur). Paper presented at *International Congress on Tropical Sericulture practices held at Bangalore*. 33.

Nagaraju, J., Noamani, M. K. R., Jolly, M. S., Datta, R. K., Vijayraghavan, Gopalkrishnan., Premalatha and Ravindrasingh (1987) MY_1 a new multivoltine strain which holds promise. *Indian silk.*, December 19–22.

Panse, V. G., and Sukhatme, P. V. (1967) Statistical methods for agricultural workers. Indian council of Agricultural Research Publication. New Delhi.

Thangavelu, K. (1988) Relative performance of pure races and cross breeds in tropical conditions of Tamil Nadu. Paper presented at *International Congress on Tropical Sericulture Practices held at Bangalore*. 47.

Yokoyama, T. (1957) On the application of heterosis in Japanese sericulture. *Proc. Internatl. Genet. Symp. Cytologia Suppl.*, 527–531.

Yokoyama, T. (1974) Utilisation of heterosis in Japanese sericulture (SABRAO Proc). *Indian J. Genet.*, **34A**: 206–210.

Yokoyama, T. (1979) Silkworm Selection and Hybridisation. (In: Genetics in relation to Insect management. Ed. Hoy, M.A. and Mokelvey, T. T.). *The Rockfeller foundation* 71–83.

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Araneidae and Theridiidae of Buxa Tiger Reserve, West Bengal: Genera *Leucauge* White, *Cyrtophora* Stoliczka and *Theridula* Emerton

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Abstract: Six araneid species *Leucauge celebesiana, L. decorata, L. pondae, L. tessellata, Cyrtophora cicatrosa* and one theridiid species *Theridula swatiae* n.sp. are reported from Buxa Tiger Reserve. *L. pondae* is a new record from West Bengal.

Keywords: Spider, Araneae, Araneidae, Theridiidae, *Leucauge, Cyrtophora, Theridula*, West Bengal.

INTRODUCTION

We have earlier reported some araneid, heteropodid and lycosid spiders of Buxa Tiger Reserve (Saha, *et al.*, 1994, '95).

Present paper deals with five species of *Leucauge* White, one species of *Cyrtophora* Stoliczka and one species of *Theridula* Emerton. *T. swatiae* is described as new to science. The species *L. pondae* Tikader is reported for the first time from West Bengal (marked * in text).

All these species are collected from Buxa Tiger Reserve and are now in the collection of Entomology Laboratory, Department of Zoology, University of Calcutta.

Leucauge celebesiana (Walckenaer)

Tetragnatha celebesiana : Walckenaer, 1841, Hist. Nat. Ins. Apt., 2: 222; Tikader, 1980, Fauna of India, Spiders: Araneae: Araneidae, Pt. 1, Zool. Surv. India, Calcutta: 83–85.

Material examined : 1 ♀, 5.IV. 1993, found in association with *Aphis gossypii* GL. (Aphididae: Aphidinae) on indet host, South Raydak, B. T. R., Jalpaiguri, West Bengal.

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***Leucauge decorata* (Blackwall)**

Tetragnatha decorata : Blackwall, 1864, Ann. Mag. nat. Hist., 14(3): 44; Tikader, 1982, Fauna of India Spiders: Araneae: Araneidae, Pt. 1, Zool. Surv. India, Calcutta: 78–80.

Material examined: 7 ♀, 5.IV. 1993, South Raydak, B.T.R., Jalpaiguri, West Bengal.

***Leucauga pondae* Tikader**

**Leucauge pondae:* Tikader, 1970, Rec. Zool. Surv. India, 64(1–4): 44–45; Tikader, 1982, Fauna of India, Spiders: Araneae: Araneidae, Pt. 1, Zool, Surv. India, Calcutta: 89–91.

Material examined: 4 ♀, 5.IV. 1993, South Raydak, B. T. R., Jalpaiguri, West Bengal.

***Leucauge tessellata* (Thorell)**

Callinathis tessellata: Thorell, 1887, Annali, Mus. civ. Genova, 25: 125; Tikader, 1982, Fauna of India, Spiders: Araneae: Araneidae, Pt. 1., Zool. surv. India, Calcutta: 80–82.

Material examined: 1 ♀, 6.IV. 1993, South Raydak, B. T. R., Jalpaiguri, West Bengal.

***Cyrtophora cicatrosa* (Stoliczka)**

Epeira (Nephila) cicatrosa: Stoliczka, 1869, J. Asiat. Soc. Beng., 33: 242; Tikader, 1980, Fauna of India, Spides: Araneae: Araneidae, Pt. 1, Zool. surv. India, Calcutta: 178–180.

Material examined: 1 ♀, 5.IV. 1993, South Raydak, B. T. R., Jalpaiguri, West Bengal.

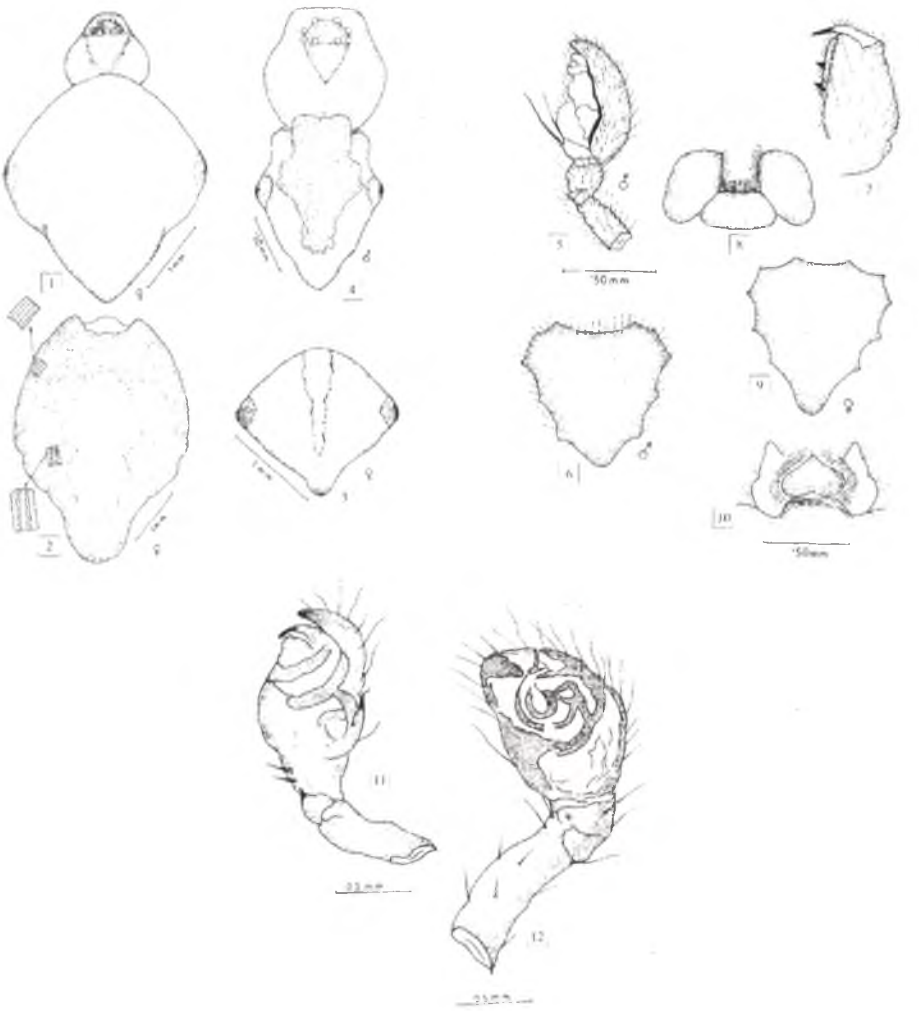
***Theridula swatiae* n. sp. (Figs. 1–12)**

Female (holotype): Measurements (in mm): Body length 4.00; carapace length 1.5, carapace width 1.1; abdomen length 2.5, abdomen width 2.1; legs as in table-1.

Cephalothorax orange–yellow, anteriorly narrowed; cephalic region raised, with faint black shades below the posteromedian eyes; fovea absent; eye-region blackish, eyes pearly–white, with black patches at bases, anterior row of eyes strongly recurved, posterior row almost straight, lateral eyes close to each other, their black patches fused; ocular quad squarish. Chelicerae brownish, broad at base, only outer margin with 2 teeth, fangs extremely short; maxillae and labium yellow, broad, flat, labium typically heart shaped, with very few brown, fine spines; legs yellow, brown–black from distal 1/3 of femur onwards; leg formula 1432.

Table 1. Length of legs of ♀ holotype of *Theridula swatiae* n.sp. (mm)

Leg	Femur	Patella & Tibia	Metatarsus	Tarsus	Total
I	0.3	1.7	0.2	0.7	2.9
II	0.2	1.0	0.2	0.4	1.8
III	0.3	1.1	0.2	0.3	1.9
IV	0.4	1.4	0.2	0.6	2.6



Figs. 1–12. *Theridula swatiae* n.sp. 1. Female, Whole body, Paratype 2. Female, Abdomen, Holotype 3. Female , Abdomen, Paratype 4. Male, Whole body, Allotype 5. Male, Palp, Allotype 6. Male, Sternum, Allotype 7. Female, Chelicerae, Holotype 8. Female, Maxillae and Labium, Holotype 9. Female, Sternum, Holotype 10. Female, Epigyne, Holotype 11. Male palp (retrolateral view), Allotype 12. Male palp (ventral view), Allotype

Abdomen orange–yellow, rhomboidal, overhanging the cephalothorax, anteriorly with a black semilunar band continuous upto the middle of abdomen, when absent (paratypes) with white patches arranged irregularly, medially with a longitudinal white band continuing upto posterior 2/3, sometimes absent, posterior 1/3 with 2 white patches submarginally, posterior tip with a black spot, besides this, juveniles/females (when black band absent) with one such on the lateral angles of the rhomboid, anteriorly produced into a hump, this antero-laterally appearing large, tuberculate, posteriorly narrowed and produced, spinnerets dorsally not visible; dorsum extremely ridged

and wavy, therefore with depressions followed by raised regions all over, with sparsely distributed white hairs, these posteriorly more evident; ventrally brown, pubescent; epigyne on either side with white patch; epigyne as in Fig .10

Male (allotype): Measurements (mm): Body length 3.0, carapace length 0.8; carapace width 1.1; abdomen length 2.2; abdomen width 2.0; legs as in table-2. Body more dark; Cephalothorax wider, anteriorly broadly narrowed; cephalic region more blackish, with scar more evident; sternum 'V' shaped but anteriorly more broad; leg formula 1432.

Table 2. Length of legs of ♂allotype of *Theridula swatiae* n.sp. (mm)

Leg	Femur	Patella & Tibia	Metatarsus	Tarsus	Total
I	1.0	2.7	0.5	1.2	5.4
II	0.5	1.3	0.3	1.1	3.2
III	0.3	1.2	0.2	1.0	2.7
IV	0.6	1.4	0.5	1.5	5.0

Abdomen with anteriorly broad, posteriorly narrowed black patch running medially upto posterior 2/3 region; each of the 3 corners of the rhomboid (laterals and posterior) with 1 black spot; abdominal hairs whitish and more evident; male palp as in Figs 5, 11, and 12; otherwise same as the holotype.

Remarks: The genus in India is a monotypic one, being represented by *Theridula angula* Tikader (Tikader, 1970) which is with cephalic region rectangular, cephalothorax posterolaterally bulbous, abdomen triangular, with two pointed spine-like shoulder humps besides the tail hump, epigyne with a stout beak-like scape. Contention of proposing the present material as a new species is because of the distinct differences with the above indicated one in the following:

1. Cephalic region never rectangular,
2. Cephalothorax posterolaterally not bulbous,
3. Abdomen rhomboidal, devoid of anterolateral spines
4. Epigyne without any beak

Etymology: The species has been named after Mrs. Swati Sengupta, who was extremely hospitable during our visit to B. T. R.

Material examined: Holotype: ♀ 5.IV. 1993, South Raydak, B. T. R., Jalpaiguri, West Bengal, India, Leg: S. Saha Allotype: ♂, otherwise data same as for the holotype; Paratype: 8 ♀s, otherwise data same as for the holotype; 2 ♀s, 4 ♂s (immature), 6.IV. 1993, found in association with *Cinara* sp. (Aphididae: Lachninae) on *Thuja* sp. (Cupressaceae), otherwise same as for the holotype.

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REFERENCES

Saha, S., Biswas, V. and Raychaudhuri, D. 1994. Heteropodidae and Lycosidae of Buxa Tiger Reserve, West Bengal. Acta arachnol., **43(1)**: 43–48.

Saha, S., Biswas, V., Majumder, S. C. and Raychaudhuri, D. 1995. Araneidae of Buxa Tiger Reserve, West Bengal, Acta arachnol., **44(1)**: 11–14.

Tikader, B. K. 1970. Spider Fauna of Sikkim. Rec. Zool. Surv. India, **64(1–4)**: 1–84.

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Short Communication

Field Performance of Plant extracts on mulberry silkworm, *Bombyx mori* L.

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Abstract: The application of petroleum ether and aqueous extracts of *Tribulus terrestris* L (zygophyllaceae) and *Psoralea coryleifolia L.* (Leguminosae) indicated that the former extended the maturity of fifth instar silkworms by 22 hrs and the latter accelerated the maturity by 6 hrs recording an increase of 12.8 and 15.1 and 4.84 percent increase in cocoon yield over control respectively. The studies indicated that the plant extracts could be cheaper and abundant source of insect hormones and can be used to increase the silk yield in commercial silkworm rearing.

Keywords: Field trial, *T. terrestris*, *P. coryleifolia*, aqueous extract, petroleum ether extract, silkworms, additional income, profit.

INTRODUCTION

Plants act as the richest source of organic chemicals on earth. Already about 10,000 secondary plant substances have been chemically defined. It is estimated that the total number of chemical may account to 4,00,000 or more (Swein, 1977). Following the discovery of "paper factor" from *Abies balsamea* Milk (Slama and Williams, 1966) insect hormones have been intensively searched in the natural flora for the past 30 years and increasing attention is being focused on the use of JH to alter the physiology of insects. Studies conducted to know the JH-mimic activity of some of the commonly occuring weed plants of South India viz., *Tribulus terrestris* and *Psoralea coryleifolia* exhibited JH–mimic activity on silkworms which extended the fifth instar larval life by a day prolonging the feeding period resulting in bigger cocoons (Rajashekhargouda, 1991). Hence a field trial was conducted to know the effect of petroleum ether extracts of *T. terrestris* and*P. coryleifolia* to benefit the sericulture community.

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A field trial was conducted in the Sericulture farm of Thiru V. Krishnaswamy, Mettupalayam, Coimbatore, India during the month of February–March, 1991 using the selected doses of petroleum ether (1000 µg/larva) as well as water extracts (10%) of both *T. terrestris* and *P. coryleifolia*. Four hundred uniform sized 48 hrs old fifth instar silkworms (PM × NB_4D_2) were cultured on M-5 leaves. The larvae were sprayed with 5 to 8 ml of the selected doses using hand atomizer and kept in dark for 30 minutes before resumption of feeding to ensure maximum absorption. Four feedings were maintained for all the treatments. Acetone control and water control were maintained for petroleum ether and water extract respectively. Observation were recorded on the larval period and cocoon yield. Economics of the above plant extract was worked out for 100 layings subtracting cost of production of additional expenditure from increased profit due to application.

E = Additional expenditure (100 layings) includes cost of extra leaf consumed due to treatment + cost of plant extract including (solvent + cost of application (acetone + spraying) + cost of labour).

I = Additional income due to increased cocoon production

Profit, P = I - E

The results of the field trial conducted to know the effects of ether and water extracts of *T. terrestris* and *P. coryleifolia* and their economics for increasing cocoon production were evaluated. The application of ether extracts of *T. terrestris* and *P. coryleifolia* extended the larval life by 22 hrs, resulting in an increase of 12.76 and 15.07 percent in cocoon yield over control. On the other hand the use of water extracts of *T. terrestris* and *P. coryleifolia* effected early maturity by 6 hrs with an increase of 4.84 per cent in cocoon yield over control.

The additional expenditure due to application of these compounds was Rs. 120 and Rs. 20 for ether and water extracts of *T. terrestris* and *P. coryleifolia* respectively. The additional income for every 100 layings was Rs. 360, 453, 173 and 173 in case of ether and water extracts of *T. terrestris* and *P. coryleifolia* respectively. The ether extracts of *P. coryleifolia* was found to be a boon giving as much as Rs. 453/100 layings. However, only Rs. 173 could be earned by use of water extracts of either *P. coryleifolia* or *T. terrestris*. The cost benefit ratio due to application of ether extracts of *T. terrestris* and *P. coryleifolia* was 1:3 and 1:4 respectively while with water extracts of both plants it was as high as 1:9 (Table 1 and 2).

Table 1. Additional cost of feeding due to application of JHs and their mimics from plant extracts

JHs and their mimics with dose	Leaf consumed on first 7 days (kg/100 dfl's)	Additional leaf consumed on 8th day (kg)	Total leaf consumed (kg)	Increase in leaf consumption (kg)
Methoprene (10 ppm)	573.4	131.6	705.0	44.2
Hydroprene (10 ppm)	580.2	136.4	716.6	55.8
PC–BK (10 µg)	577.9	133.6	711.5	50.7
PC–BME (10 µg)	582.4	141.2	723.6	62.8
T.terrestris (1000 µg)	575.7	140.4	716.1	52.3
P. coryleifolia(1000 µg)	579.0	134.0	713.0	52.2
Control	660.8	*	660.8	*

* No observation as the larvae attained the pupal stage

Table 2. Economics of the use of plant products for increasing cocoon production of *B. mori* L.

Plant extract with dose	Cocoon weight (g)	ERR (%)	Cocoon production (kg/100 dfl's)	Per cent increase over control (%)	Extra leaf consumed (kg/100 dfl's)	Additional expenditure due to application (/100 dfl's) (Rs)	Additional income/100 dfl's (Rs)	Net profit /100dfl's (Rs)
T. terrestris (PE) (1000 µg)	1.5	78	45.2	12.8	52.3	120	480	360
P. coryleifolia (PE) (1000 µg)	1.5	78	46.2	15.1	52.2	120	573	453
T. terrestris (WE) 10%	1.3	80	41.7	4.8	–	20	193	173
P. coryleifolia (WE) 10%	1.3	80	41.7	4.8	–	20	193	173
Acetone control	1.3	80	40.1	–	–	–	–	–
Water control	1.2	80	39.8	–	–	–	–	–
Absolute control	1.3	80	40.4	–	–	–	–	–

PE = Petroleum ether extract
WE = Water extract

The additional income by use of petroleum ether extracts of *T. terrestris* and *P. coryleifolia* was Rs. 360 and 453 respectively while with water extracts it was only Rs. 173/100 layings. However, according to Benchamin *et al.*, (1983) the net extra income due to application of methoprene (manta) was approximately Rs. 85.00 per 100 layings which is far less compared to the profit recorded in the present study.

Among the solvent extracts and water extracts the latter was more suitable for the application, incurred only an additional expense of Rs. 20 while the net profit was Rs. 173 for every 100 layings brushed. Interestingly, the application of water extract shortened the fifth instar larval duration by 6 hrs without bringing down the effective rate of survival. From the application point of view, preparation of water extract of *P. coryleifolia* is easy which needs no skill. It could be inferred from the above investigation that even the weeds considered as biological wastes instead of being unutilised can be very easily exploited by sericulturists to improve silk and egg yield of *B. mori* for commercial silk and basic seed production respectively. Further, these renewable natural resources of the tropics particularly in a developing country like India can go a long way as a component of low cost production technology making the environment pollution free which forms an ecologically sound technology.

REFERENCES

Benchamin, K. V., Jolly, M. S., Benjamin, D. A. I. and Ratnasen. (1983) On the commercial application of juvenile hormone Analogue "Manta" in silkworm *Bombyx mori L.* and the economics. p 73. In: National seminar on silk research and development. Central Silk Board, Bangalore.

Rajashekhargouda, R. 1991. Studies on methods to increase silk yield of *Bombyx mori* Ph.D. thesis submitted to Tamil Nadu Agricultural University. 243.

Slama, K. and Williams, C. M. (1996). The juvenile hormone V. The sensitivity of the bug *Pyrrhocoris apterus* to a hormonally active factor in American paper pulp. *Biological Bulletin.* **130**: 235–246.

Swein. (1977). Secondary plant compounds of protective agents. *Ann. Rev. plant physiol.* **28**: 479–501

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Short Communication

Quantitative loss due to Nut Crinkler (*Paradasynus rostratus* Dist.) Damage on Coconut

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Abstract: Studies were conducted at the College of Agriculture, Vellayani to assess the damage and quantitative loss inflicted by the nut crinkler *Paradasynus rostratus* Dist. The results showed that feeding by the coreid bug reduced the weight and volume of nuts, weight of copra and oil. Correlation studies showed that there was significant negative correlation between area damaged on the pericarp (husk) and other nut parameters in the infested nuts.

Keywords: Nut crinkler, *Paradasynus rostratus*, extent of damage

INTRODUCTION

The nut crinkler *Paradasynus rostratus* Dist. has gained notoriety as a serious pest of coconut causing economic damage. The bug was reported as a pest of coconut by Kurien *et al* (1972) and it is at present observed in varying intensities in Kerala. The biology of this bug on coconut has been studied by Kurien *et al* (1972). Both the adults and nymphs damage the nuts by their feeding, but precise information on the effect of coreid bug feeding on the nut is lacking. Hence studies were conducted at the College of Agriculture, Vellayani to assess the damage caused by the coreid bug to the nuts and the quantitative loss inflicted.

Coconut palms of variety WCT (West Coast Tall) of uniform age and height infested by coreid bug were selected for the studies. Thirty five bunches of coconut consisting of healthy as well as damaged nuts were selected and harvested. From each bunch, the damaged and healthy nuts were cut separately and tagged. Then, the area damaged on the pericarp of the nut was measured by mapping the area using a graph paper and expressed in square millimeters. The quantitative characters of the healthy and damaged nuts studied, included weight of the whole nut (g), volume of the nut (cc). The kernels were removed, dried and weight of copra (g) was recorded. The oil was extracted from the copra of each nut separately and weight recorded. The percentage of oil in the copra of each nut was then worked out. All these characters were studied in the

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unaffected healthy nuts of the same bunch and correlations were worked out between the area damaged and the percent increase/decrease of the quantitative characters of the affected nuts in comparison to unaffected nuts.

When the tender buttons are attacked by the bugs by their feeding through the perianth by inserting their stylets, button fall often results. Those which remain on the bunch, even after the attack, show feeding punctures as eyelike spots in the early stages. Later on as the damaged buttons grow, the eye like spots develop to deep furrows and crinkle with severe gummosis on the husk. Due to feeding damage and toxaemia, the internal contents of the nuts are also affected.

Table 1. Effect of feeding of *Paradasynus rostratus* on the size and weight of coconut and on the yield of copra and oil

Nut characters	Healthy (Mean values)	Infested	Mean % loss
Area of pericarp damaged (sq.mm)	–	2387	–
Weight of nut with husk (g)	1401	1149	17.99
Volume of nut with husk (mm^3)	1874	1500	19.96
Weight of nut without husk (g)	391	318	18.67
Volume of nut without husk (mm^3)	330	248	24.78
Weight of copra (g)	106	81	23.58
Weight of oil (g)	72	49	31.94
Percentage of oil	67	58	13.40

The data in Table 1 give a clear picture of the effect of feeding of the coreid bug on the traits of the coconut. The mean area of pericarp damaged per nut was 2387 mm^2. The mean weight and volume of the infested nut with husk was lesser compared to that of the healthy nut. The mean percentage loss in the weight and volume of the whole infested nut with husk was 17.99 and 19.96 respectively compared to the healthy nut. The same trend was observed in the dehusked nut. The mean percentage loss with regard to weight and volume of nut was 18.67 and 24.78 compared to the healthy nut. The mean weight of copra and oil per infested nut were 81.0 and 49.0 g respectively, compared to 106.0 g and 72.0 g in the healthy nut. The percentage loss in weight of copra and oil due to the damage by this bug were to the tune of 23.58 and 31.94 respectively.

The results on the correlation studies are presented in Table 2. The studies show that as the area of damage increased, there was corresponding decrease in the weight of dehusked nut, volume of dehusked nut, weight of copra, weight of oil and oil content. There was significant negative correlation between the area damaged and these parameters observed, the *r* values being -0.26, -0.22, -0.30, -0.35 and -0.42 respectively for the characters. This quantitative loss may be due to the toxaemia produced by the feeding of the bugs which affect the water transport mechanism leading to reduction

Table 2. Correlation coefficient between the area of pericarp damaged (sq. mm) by coreid bug and nut characters

Extent of damage	Nut characters	*r* value	Inference
Area of pericarp damaged (sq. mm)	× Weight of whole nut (g)	0.01	NS
do	× Volume of nut ($\bar{c}\,\bar{c}$)	-0.12	NS
do	× Weight of dehusked nut(g)	-0.26	**
do	× Volume of dehusked nut ($\bar{c}\,\bar{c}$)	-0.22	**
do	× Weight of copra (g)	-0.30	**
do	× Weight of oil (g)	-0.35	**
do	× Oil content (%)	-0.42	**

** Significant at 1% level

in weight of the nut, the endocarp vis-a-vis copra and oil content.

REFERENCES

Kurian, C., Abbraham, V. A. and Abdulla Koya, K. M. (1979). Studies on *Paradasynus rostratus* Dist. (Heteroptera: Coreidae) a pest of coconut. *Proc. 2nd Ann. Symp. on Plantation Crops*. 484–503.

Kurian, C., Pillai, G. B. Abraham, V. A. and Mathen, K. (1972). Record of a coreid bug (nut crinkler) as a new pest of coconut in India. *Curr. Sci.* **12**: 37.

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Short Communication

Mass Rearing of American Serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)

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Abstract: American serpentine leaf miner, *Liriomyza trifolii* (Burgess) is mass reared under insectary condition. Cowpea, *Vigna sinensis* (L.) served as the best host. This technique is found to be cost effective and very well suited to the Indian conditions. The same aged adults can be obtained by using this technique.

Keywords: Mass rearing, serpentine leaf miner, *Liriomyza trifolii*

American serpentine leaf miner, *Liriomyza trifolii* (Burgess), native of Florida (USA) has been accidentally introduced into India during 1990–91, along with chrysanthemum cuttings imported from United Kingdom. *L. trifolii* which is a serious polyphagous pest in USA and many European countries is now getting its hold in India. Since there is no standard mass rearing technique for this serious polyphagous pest is available, the studies were taken up to design cost effective and efficient mass culturing technique.

Eventhough *L. trifolii* is a polyphagous pest known to infest a wide range of crops, observations revealed cowpea, *Vigna sinensis* (L.) as one of the best alternative host. Hence, cowpea was selected as a host for mass culturing *L. trifolii* under insectary-conditions.

The seeds of cowpea (cv. CO6) were sown in polyethene bags (15×6 cm) and kept inside oviposition cage (45×45×60 cm) at rate of 20 plants per cage. For initiation of mass culture the adults of *L. trifolii* were collected from field using test tubes or small glass vials. On 20 days after sowing (DAS) of cowpea the field collected adults were released at the rate of 10–15 pairs per cage.

The adults laid eggs in cowpea plants, from which the larvae hatched and developed within a week. The prepuparial larva (the stage of larva just prior to pupation), usually comes out of the mine and pupates in soil. The larval period ranges from 4–6 days, which will be completed in the leaf mine. The emerging prepuparia were collected by any of the following ways.

A tray (60×40×10 cm) was uniformly filled with finely ground and sieved (200 mesh) black cotton coil, as a thin layer of 1–2 mm. The infested plants with leaf

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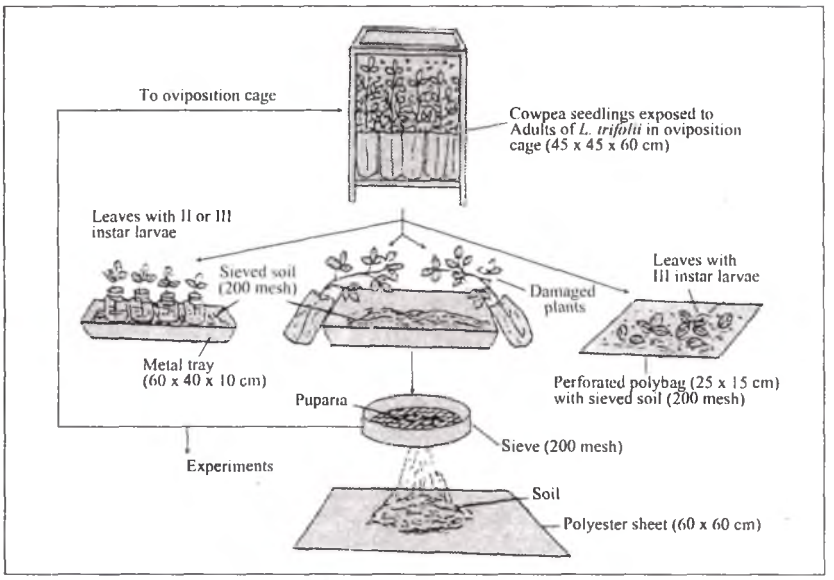

Fig. 1. Mass culturing of serpentine leaf miner – Flow chart

mines were kept around this tray in a slanting position (Fig. 1), such that the infested leaves with larvae are towards the middle of the tray. This is to facilitate the prepuparia coming out of mines to fall in the soil layer for pupation.

In the second method the trifoliate leaves with II instar larvae were plucked along with petiole. The petiole of the leaves were inserted with cotton plug into glass vials filled with water and a drop of glycerine to ensure the turgidity of leaves. Such vials were kept inside same type of tray, as used in the I method at the rate of 12–15 vials per tray in a spaced manner. The prepuparia coming out of mines would fall in the soil layer and enter pupation.

The puparia from III instar larvae can be collected by the following method. Here the polyethene bag (25 × 15 cm) were kept in a flattened condition with a thin layer (1–2 mm) of sieved black cotton soil (200 mesh) on the lower side, with perforations on the upper side. The individual leaves with late stages of III instar larvae were plucked and spread on the soil layer inside the polyethene bag. The polyethene bag was then sealed with staples. Since, the III instar larval period is very short, prepuparia would fall in soil and pupate within 1–2 days before the complete drying of leaves.

Thus by following the above three methods the leaf miner puparia were collected in soil. They can be separated by sieving the soil with puparia (orange yellow in colour), through 200 mesh sieve. The puparia were stored in test tubes or in small glass vials upto the emergence of adults (approximately 7–9 days), and were either used or recycled in mass culturing.

In this mass culturing technique cowpea was selected as a host, because of its bushy foliar growth in a short period of time under laboratory conditions, and it was found to be attacked heavily by *L. trifolii*. But, Vercambre (1980) used *Phaseolus vulgaris* for mass rearing *L. trifolii* under laboratory conditions.

This method of mass rearing developed here is cost effective, which can be done with locally available material. The other advantage lying with this method is uniform aged puparia were obtained by following latter two methods of puparial collection. This resulted in uniform emergence of adults, which can be used in experiments accordingly.

The main disadvantage of this method is that the adults cannot be stored alive for long time, however, by providing honey solution it can be stored alive for a period of 2–3 days.

REFERENCE

Vercambre, B. 1980. Studies conducted in Reunion Island on the broad-bean leaf miner, *Liriomyza trifolii* (Burgess). Reveue–Agricole–et Sucriere–de–1, Ile–Maurice. **59(3)**: 147–157.

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Short Communication

Effect of different density of eggmass of top borer (*Scirpophaga excerptalis* Walker) on growth, yield and quality of sugarcane

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Abstract: Field experiment to determine the influence of eggmass density of top borer (*Scirpophaga excerptalis* Wlk.) on growth, yield and quality of sugarcane was studied at G. S. Sugarcane Breeding and Research Institute, Seorahi in 1991–92 and 1992–93. The incidence of top borer during 3rd and 4th brood varied from 8 to 14% and 5 to 13%, respectively. The crop affected by the pest during the third brood suffered more as compared to fourth brood. For example, the incidence during 3rd brood accounted for about 34–48% reduction in height, 44–57% in stalk weight, 0.66–1.53% in sucrose, 0.90–2.55 in purity and 39 to 55% in CCS MT per ha, whereas during the 4th brood it accounted for about 18–22%, 19–30%, 0.07–0.91, 0.29–2.25 and 39–51%, respectively. The overall reduction in sugarcane yield due to top borer varied from 36 to 51%.

Keywords: *Scirpophaga excerptalis* Walker, density, yield sugarcane

INTRODUCTION

The top borer (*S. excerptalis* Wlk.), a serious and destructive pest of sugarcane completes four to seven broods in a year in various parts of India. Of these broods, the third one is most destructive as it coincides with the commencement of grand growth phase of sugarcane (Kalra and Chaudhary, 1964a). Gupta (1959) reported a loss of 18.5 t/ha in yield due to this pest at 55% incidence, besides deterioration in the quality of sugarcane (Rajani, 1960; Gupta *et al.*, 1965). In the present communication, attempts have been made to study the effect of eggmass density of top borer on the growth, yield and quality of sugarcane.

Field experiment with CoS 802 variety of sugarcane was conducted at G. S. Sugarcane Breeding and Research Institute, Seorahi, Kushinagar in autumn season of 1991–92 and 1992–93. There were six treatments, viz., artificial inoculation with 2, 3, 4 and 6 eggmasses/row, natural oviposition and eggmass removal during 3rd and 4th brood of top borer, each replicated four times in a randomised block design.

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Table 1. Effect of eggmass density of top borer on incidence, growth, yield and quality of sugarcane

Sl. No.	Treatment	Third Brood						Fourth Brood						Yield Mt/ha
		Top borer incidence	Height (CM)	Weight (gms)	CCS (Mt/ha)	Sucrose %	Purity coefficient	Top borer Incidence	Height (CM)	Weight (gms)	CCS (Mt/ha)	Sucrose %	Purity coefficient	
1.	Stapling of 2 eggmass/ row (3174 eggmass/ha)	6.56	105.95	394.87	3.92	16.01	84.27	5.05	129.30	511.75	3.99	16.66	86.27	33.80
2.	Stapling of 3 eggmass/ row (4761 eggmass/ha)	7.75	106.97	384.62	4.31	15.88	85.48	6.15	131.40	538.25	4.30	15.84	85.15	37.50
3.	Stapling of 4 eggmass/ row (6349 eggmass/ha)	9.27	95.0	362.62	3.45	15.14	80.17	8.61	125.05	466.25	4.06	16.37	84.31	34.32
4.	Stapling of 6 eggmass/ row (9523 eggmass/ha)	13. 83	83.15	299.87	3.17	15.20	83.83	12.68	127.12	484.37	3.42	16.40	85.37	28.84
5.	No eggmass Stapling	11.17	85.95	333.12	3.56	15.90	84.92	10.15	127.15	509.37	3.54	15.82	85.60	30.98
6.	Removal of eggmass	3.42	159.37	700.37	7.06	16.67	86.38	2.68	159.47	622.00	7.00	16.73	86.56	58.45
	S.E	1.12	12.25	73.35	0.53	0.71		0.92	8.61	53.88				6.26
	C.D. 5%	2.39	26.12	156.31	1.13	1.53	NS	1.97	18.34	114.83	NS	NS	NS	13.36

In order to record the per cent incidence, sugarcane plants affected by top borer during 3rd and 4th broods were tagged separately. Ten such plants from each brood were harvested and height (base to joint), stalk weight, sucrose, purity coefficient and CCS MT/ha were determined.

Inoculation of eggmass in increasing doses promoted the incidence of top borer significantly, being the maximum of 13.83% in 3rd brood and 12.68% in 4th brood due to inoculation of 6 eggmasses (Table 1). This treatment recorded significantly more incidence than the natural infestation level of top borer. The removal of eggmass decreases the incidence by 3.44 and 2.68 per cent in 3rd and 4th brood, respectively, and hence is considered useful in minimising the incidence of top borer.

Natural incidence of top borer artificially created by placing eggmasses in different numbers decreased the height and weight of stalk significantly at 3rd as well as 4th brood as compared to treatment where eggmass removal was practised (Table 1). The magnitude of reduction in both the attributes due to different density of eggmasses was statistically on par, indicating thereby that the presence of even 2 to 3 eggmass can affect the growth of sugarcane to an alarming level.

The decrease in sugarcane yield and CCS due to different treatments followed more or less similar trend as observed in the case of growth (Table 1).

The incidence of top borer varied from about 8 to 14% during 3rd brood which accounted for about 33 to 48% reduction in height, 44–57% in stalk weight, 0.66 to 1.53% in sucrose content, 0.90 to 2.55 in purity coefficient and 39 to 55% in CCS t/ha as compared to the removal of eggmass treatment. Similarly, the incidence of top borer from about 5 to 13% during 4th brood accounted for about 18 to 22, 19 to 30, 0.07 to 0.91, 0.29 to 2.25 and 39 to 51% reduction in these attributes, respectively. This pest reduced the yield by 36 to 51%. These results, thus, led to conclude that the incidence during 3rd brood is more harmful than the 4th brood. The infestation of this pest at either stage may damage the growth, yield and quality of sugarcane to an alarming extent as already reported by Kalra and Chaudhary (1964a), Agarwala and Prasad (1956) and Rajani (1960).

REFERENCES

Agarwala, S. B. D., and Prasad, S. N. (1956) The status of sugarcane top borer *Scirpophaga nivella* Fabr. in Bihar: I. Comparative studies on the damage caused by each brood of the pest. *Indian Sugar*, **6**: 60–66.

Gupta, B. D. (1959) Insect pests of sugarcane in India–III. The top borer, *Scirpophaga nivella* Fabr. *Indian Sugar*, **9**: 127–149.

Gupta, K. M., Singh, B and Sagar, G. (1965) Estimation of losses caused by top borer (*Scirpophaga nivella* F.) to sugarcane crop in Uttar Pradesh. *Indian Sugar*, **15**: 101–106.

Kalra, A. N. and Chaudhary, J. P. (1964a) Assessment of losses by sugarcane pests–Loss caused by top borer of sugarcane. *Indian. J. Sug. Cane Res. Dev.*, **8**: 261–264.

Rajani, V. G. (1960) Estimation of losses caused by insect pests of sugarcane crop in Uttar Pradesh. *Proc. Bienn. Conf. Sug. Cane Res. Dev. Wkrs.* India, **4**: 476–487.

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Short Communication

New Host Records of the almond Moth, *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae) in South India

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Abstract: Almond moth, *Ephestia cautella* (Walker) has been successfully reared on decorticated seeds of *Simarouba glauca* DC (Simaroubaceae) and sunflower cake following natural infestation on these hosts found in the market and storage places around Bangalore. The insect took 30-35 days on sunflower cake and 35-55 days on *S. glauca* to complete its development from egg to adult. It suffered 18-20% larval mortality on seeds of *S. glauca*.

Keywords: New host record, *Ephestia cautella* (Walker) sunflower cake, *Simarouba glauca*

The cosmopolitan almond moth, *Ephestia cautella* (Walker) affects a number of stored grains and dried fruits. These include wheat (*Triticum aestivum*), bajra (*Pennisetum americanum*), maize (*Zea mays*), various pulses (Chaudary and Bhattacharya, 1976), sorghum (*Sorghum bicolor*), rice (*Oryza sativa*), sesame (*Sesamum indicum*) (Mookherjee *et al.*, 1969), groundnut (*Arachis hypogea*) (Khan and Selman, 1980; Mejule and Onykuie, 1980; Mookherjee *et al.*, 1969), almonds, dried fruits (Cox, 1975), chocolates, biscuits, confectionaries etc. (Pruthi and Singh, 1950). It is also known to attack some of the unusual foods such as milk powder (Narafu *et al.*, 1976), garlic (Bharadwaj and Thakur, 1974), onion (Lal and Varma, 1975), dried chillies (Joshi, 1975; Ramzan and Darshan Singh, 1983), castor (Pattinson, 1969), copra (Anon., 1974), dried mushroom (*Morchella esculenta*) (Srinath and Gill, 1975), even hides and wool (Knoche, 1961). The insect also has been reported to attack seeds of sunflower in Russia (Atanasov, 1974) and Australia (De Laney, 1978).

Recently we observed severe infestation by this insect on sunflower cake and decorticated seeds of *Simarouba glauca* DC (Simaroubaceae) a potential oil seed tree being popularised by the University of Agricultural Sciences, Bangalore.

The sunflower cake sampled for studies on some of the preservatives such as sodium benzoate to prevent mould revealed the presence of larvae of *E. cautella*. These samples were collected from the Bangalore market. The larvae were reared to adults and the second generation was reared for finding out the suitability of the food for the insect.

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Decorticated seeds of *S. glauca* which were stored in the vicinity of groundnut were infested by the larvae of *E. cautella*. The adults emerging from these were released on fresh decorticated seeds of *S. glauca* to find out whether the insect successfully breeds on them or not.

E. cautella successfully completed two generations both on sunflower cake and seeds of *S. glauca* after which the study was discontinued. The developmental period (from egg to adult emergence) was shorter on sunflower cake wherein it lasted for 30-35 days compared to 35-55 days in the case of *S. glauca*. Several third instar larvae (18-20%) were found dead in *S. glauca* which was not observed in the case of sunflower cake. The developmental periods observed during this study are quite comparable with those reared on groundnut which lasted for 33.85, 36.71, 47.96 and 59.00 days at 35°, 30°, 25° and 20°C at Bangalore (Ravi Prasad, 1988). Thus both sunflower cake and the seeds of *S. glauca* are suitable hosts for the development of the insect. *E. cautella* has not been recorded on these hosts so far in India. However, as mentioned earlier, it has been reported to attack seeds of sunflower in Australia (De Laney, 1978) and Russia (Atanasov, 1974).

As in the case of other hosts the larvae webbed pieces of sunflower cake and seeds of *S. glauca* and fed on them. A sheet of silken mat was formed with the increased population of the insect on both the hosts.

Though the market collected sunflower cake at the beginning contained infestation by both *E. cautella* as well as *Oryzaephilus mercator* (Coleoptera: Silvanidae) with the multiplication of *E. cautella, O. mercator* was completely replaced. However, when *E. cautella* was excluded, a good colony of *O. mercator* developed on sunflower cake.

REFERENCES

Anonymous, 1974. Report to the Government of Gilbert and Ellice Island colony on a survey of insect pests of stored crops. U. N. D. P. Report No. 3246.

Atanasov, K. 1974. Insects attacking stored sunflower seeds. *Rastit. Zash.*, **22 (4)**: 17–21.

Bharadwaj, A. K. and Thakur, J. R. 1974. *Ephestia cautella* infesting stored garlic (*Allium sativum*). *Curr. Sci.* **43**: 419–421.

Chaudary, R. R. P. and Bhattacharya, A. K. 1976. Larval developmental behaviour of *Ephestia cautella* (Wlk) on several food commodities. *Bull. grain Tech.* **14**: 1–8.

Cox, P. D. 1975. The suitability of dried fruits, almonds and acarobs for development on *Ephestia figuliella, E. cautella* and *E. calidella*. *J. stored Prod. Res.* **11 (3/4)**: 229–233.

De Laney, N. E. 1978. Sunflower growing - a changing industry. *Queensland Agric. J.* **104**: 269–278.

Joshi, G. 1975. *Ephestia cautella* infesting a new host: red hand ground chillies (*Capsicum frutescens*) in India with notes on the habit of larvae. *Z. angew. Zool.* **62**: 243–248.

Khan, A. R. and Selman, B. J. 1980. On the use of peanut as an ideal culture medium for *Cadra cautella*, a pest of date fruits in Egypt. *Agric. Res.* **54-(1)**: 127–131.

Knoche, C. 1961. *Ephestia cautella* (WLK) as a pest of hides of peccaries and hairy sheep from Brazil. *Anz. Schadlingsk.* **34 (9)**: 141–143.

Lal, S. S. and Varma, B. K. 1975. Attack of *Cadra cautella* in stored onions at Hyderabad. *Bull. grain Tech.* **12**: 238–239.

Mejule, F. O. and Onykule, P. 1980. Survey of large stacks of groundnut for insect infestation in Kano. *Rep. Nigerian Stored Prod. Res. Inst.* No. 6: 66–73.

Mookherjee, P. B., Bose, B. N. and Singh, G. 1969. Some observations on the damage potential of the almond moth, *Cadra cautella* in eight different stored grains. *Indian J. Ent.* **31**: 1–6.

Narafu, T., Takahashi, H. and Takahashi, T. 1976. Investigations on the ecology of injurious insects in milk powder. *Bull. Aichi Inst. Public Health*, **17 (4)**: 62–69.

Pattinson, I. 1969. The National Agricultural products Board Proceedings Part 2. Storage problems. *Trop. stored Prod. Inf.* **17**: 23–31.

Pruthi, H. S. and Singh, M. 1950. Pests of stored grain and their control. I. C. A. R. Pub. No. **5**, xvii, 15–38.

Ramzan, M. and Darshan Singh, 1983. Record of *Ephestia cautella* from chilli at Ludhiana. *J. Bombay nat. Hist. Soc.* **79**: 434.

Ravi Prasad, T. N. 1988. Studies on bioecology of *Ephestia cautella* (Walker) (lepidoptera: Phycitidae) infesting stored groundnut and its management. M. Sc. (Agri.) thesis. University of Agricultural Sciences, Bangalore.

Srinath, D. and Gill, J. S. 1975. Outbreaks and new records. *Pl. Prot. Bull.* **23 (2)**: 49–54.

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Short Communication

Concentration of Creatine and Creatinine in the Haemolymph of the Sixth Instar Larva of *Orthaga exvinacea* Hampson during Development

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Abstract: The presence of creatine and creatinine from insect haemolymph has rarely been reported. The finding of the presence of creatine and creatinine in substantial amount in the haemolymph suggests that both are important nitrogenous compounds of the haemolymph. The functional role of creatine and creatinine are suggested.

Keywords: *Orthaga exvinacea*, creatine, creatinine, haemolymph.

INTRODUCTION

The phosphagen theory of Baldwin (1967) holds the general assumption that invertebrates contain phosphoarginine instead of phosphocreatinine. Ennor and Morrison (1958) in a review on the distribution of phosphocreatine in marine organisms, have concluded that creatine is a phosphagen characteristic of invertebrates. Eventhough the presence of creatine and creatinine has been demonstrated in insect excreta, their presence in insect haemolymph has rarely been reported (Lazar and Mohamed, 1991).

The larva of *Orthaga exvinacea* Hampson (Pyralidae: Lepidoptera) was reared on their natural food, mango leaves (*Mangifera indica*) under laboratory conditions. The chronogically comparable final instar larvae were separated from the colony and reared in beakers with limited numbers. The analysis of the haemolymph of the sixth instar larva was undertaken during the period of development with an interval of 24 hrs. This period was started from 0 h (just after moulting and initiation of feeding) to 120 h (pupal state). The haemolymph was collected from the larva and deprotenized with 2/3 N sulphuric acid and 10% sodium tungstate. Aliquots of the supernatant were analysed for creatine and creatinine according to MacFate *et al.*, (1954).

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The change in the concentration of creatine and creatinine in the haemolymph of the larva during development are presented in Table 1 and Table 2 respectively.

Table 1. Creatine content of haemolymph

Larval period	Creatine, Mean ± SD	
	μ mole/ml	μ mole/total tissue
0 h	0.22±0.04	0.003±0.001
24 h	0.23±0.04	0.004±0.001
48 h	0.27±0.05	0.009±0.002
72 h	0.17±0.03	0.007±0.001
96 h	0.16±0.04	0.006±0.001
120 h	0.18±0.03	0.002±0.003

Five samples each were used in determinations. The creatine content per total volume of tissue were calculated based on the data of Kuzhivelil (1991)

Table 2. Creatinine content of haemolymph

Larval period	Creatinine, ± SD	
	μ mole/ml	μ mole/total tissue
0 h	0.19±0.03	0.003±0.0005
24 h	0.22±0.04	0.004±0.0007
48 h	0.27±0.04	0.009±0.0013
72 h	0.39±0.06	0.016±0.0025
96 h	0.26±0.04	0.009±0.0014
120 h	0.12±0.02	0.001±0.0002

Five samples each were used in determinations. To calculate the creatinine content per total volume of haemolymph the data of Kuzhivelil (1991) were used.

The creatine per unit volume was increased from 0 h to 48 h and hosed the highest value. From 72 h the concentration was decreased to the lowest value at 96 h. At 120 h the concentration was slightly increased. The concentration of creatinine per unit volume of haemolymph was increased from 0 h to 72 h which recorded the highest value. Later, the level was sharply decreased and the lowest value was recorded at 120 h.

The present finding that the larval haemolymph contains both creatine and creatinine in considerable amounts supports the studies of Roche *et al.*, (1957) and Lazer and Mohamed (1991). The amount found in the haemolymph, 0.16 to μ mole/ml creatine and 0.12 to 0.39 μ mole/ml creatinine can be compared to those found in the larva of *Spodoptera mauritia*, 0.16 to 1.46 μ mole/ml creatine and 0.42 to 2.70 μ mole/ml creatine (Lazar and Mohamed 1991). The results suggest that creatine and creatinine are important compounds in the haemolymph. But the functional significance of these materials other than osmoregulatory is not certain in the larva. In vertebrates, where

these compounds are found, it is used as a phosphagen in muscle metabolism. But in the larva no such anabolic activity is taking place in the feeding periods of the instar. Another possibility is that these compounds can arise as a form of waste nitrogen. It is possible that phosphocreatine may by used as storage form of energy in the larval metabolism. Comparatively high amount of these compounds found in the early stages of the instar supports the above view.

REFERENCES

Baldwin, E. (1967) *Dynamic Aspects of Biochemistry* V Edition Cambridge University Press, London.

Ennor, A. H. and Morrison, J. F. (1958) Biochemistry of the phosphagen and related guanidines. *Physiol. Rev.*, **38** 613–674.

Lazar, K. V. and Mohamed, U. V. K. (1991) Ontogenic changes in the titre of creatine and creatinine in the larval haemolyemph of the moth *Spodoptera mauritia* Boisduval. *Comp. physiol. Ecol.*, **6**: 97–99.

MacFate, R. P., Cohn, C. M. D., Eichelberger L. and Cooper, J. A. D. (1954) Symposium on azotemia. *Am. J. Clin. Path.*, **24**: 511–571.

Kuzhivelil, B. T. (1991) Studies on nitrogen metabolism in *Orthaga exvinacea* Hampson (Pyralidae: Lepidoptera). Ph.D. Thesis, University of Calicut, Kerala, India.

Roche, J., Thoai, N. V. and Robin, Y. (1957) Sur la presence de creatine chez les invertebres et sa signification biologique. *Biochem. Biophys. Acta.*, **24**: 514–519.

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